

Investigation of malignant hyperthermia in Sweden

Eva Ranklev Twetman



Lund 1986

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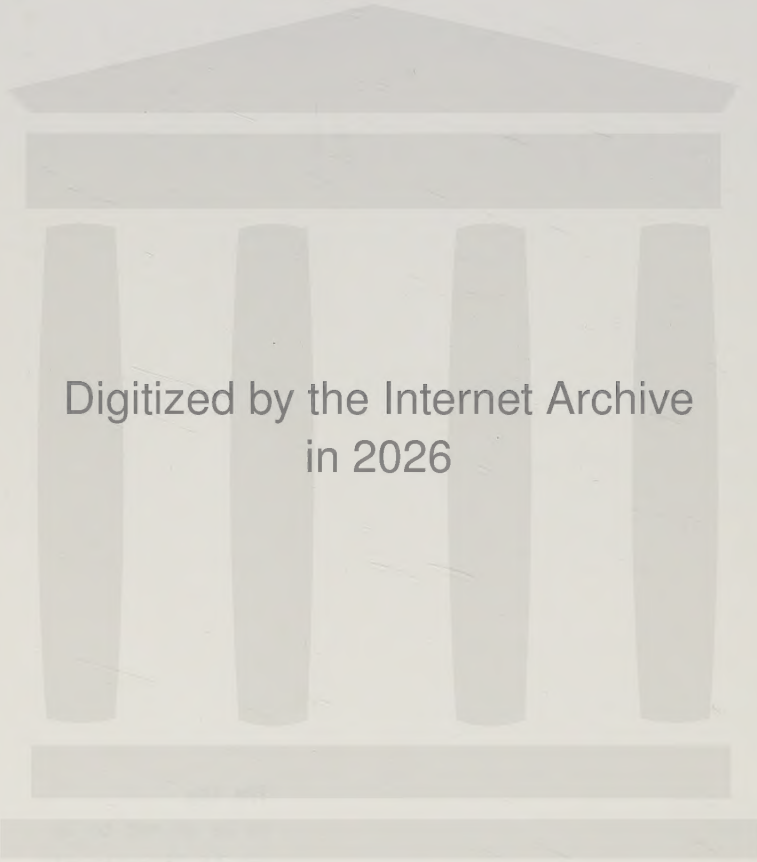
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The key
to be or not to be
may be "hypertermi"

T. Twetman



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This thesis is based on the following papers. They are referred to in the text by their Roman numerals.

- I. Ørding, H., Ranklev, E., and Fletcher, R. (1984). Investigation of malignant hyperthermia in Denmark and Sweden. *Br. J. Anaesth.* 56, 1183-1189.
- II. Ranklev, E., and Fletcher, R. Investigation of malignant hyperthermia in Sweden. Submitted for publication in *Acta Anaesthesiol. Scand.*
- III. Ranklev, E., Fletcher, R., and Blomquist, S. (1986). Static v. dynamic in vitro tests in the diagnosis of malignant hyperthermia susceptibility. *Br. J. Anaesth.* in press.
- IV. Ranklev, E., Monti, M., and Fletcher, R. (1985). Microcalorimetric studies in malignant hyperpyrexia susceptible individuals. *Br. J. Anaesth.* 57, 991-993.
- V. Ranklev, E., Henriksson, K-G., Fletcher, R., and Germundsson, K. Clinical and muscle biopsy findings in relation to malignant hyperthermia susceptibility. Submitted for publication in *Acta Neurol. Scand.*

CONTENTS

Introduction	7
The present investigation	20
Patients and Methods	20
Results	32
Discussion	47
General conclusions	57
Acknowledgements	58
References	60
Paper I	71
II	79
III	93
IV	101
V	105

INTRODUCTION

Malignant hyperthermia (MH) is a rare pharmacogenetic disorder characterized by an often fulminant hypermetabolic response to anaesthetic drugs. Without treatment fulminant MH is fatal. In early reports the mortality rate was high, but with increasing awareness of the disease and the need for prompt treatment it is now less than 10% (Pollock and Britt, 1983). Estimations of the incidence of MH vary between one in 14 000 (Britt and Kalow, 1970 a) and one in 200 000 anaesthetics (Ellis and Halsall, 1980). According to Ørding (1985) suspicion of MH is raised in one anaesthetic of 16 000, but the incidence of fulminant MH is only one in 250 000 anaesthetics. MH occurs mostly amongst children, teenagers and young adults.

As malignant hyperthermia susceptibility (MHS) is an inherited disorder (Ellis and Halsall, 1980) screening is indicated in families where a member has had an MH reaction.

History

The first well documented report of malignant hyperthermia was described by Denborough and Lovell in 1960. A complete study of this family, in whom ten deaths under ether anaesthesia had occurred, was published in 1962 (Denborough et al., 1962). These publications were later followed by a flood of case reports of MH mostly from North America, Australia, South Africa and Great Britain. The clinical features of MH which gradually emerged from the different case reports were comprehensively illustrated in a statistical review by Britt and Kalow (1970a).

In 1968, a leader in British Medical Journal forwarded the suggestion that the hyperthermia was due to increased muscle metabolism in association with some form of congenital myopathy. The findings of highly elevated creatine phosphokinase (CK) levels 24 hours after an MH reaction (Denborough et al., 1970b), raised resting CK levels in family members of known MH patients (Isaacs and Barlow, 1970) and clinical myopathy in association with MH (Denborough et al., 1970a) further confirmed this belief.

The finding that the Landrace pig suffered from a syndrome identical to human MH led to demonstrations of the biochemical changes that occur before and during the porcine MH reaction (Hall et al., 1966; Harrison et al., 1968). Investigations of these animal models also led to the identification of triggering agents, as well as therapeutic trials with procainamide (Strobel and Bianchi, 1971), procaine (Harrison, 1973) and dantrolene (Harrison, 1975).

The malignant hyperthermia syndrome

MH develops in susceptible individuals in response to a wide variety of drugs, most commonly the potent inhalational anaesthetics, and suxamethonium. The onset of porcine MH is usually more abrupt when suxamethonium is used either alone or in conjunction with inhalational anaesthetics (Gronert, Milde and Theye, 1976; Gronert and Theye, 1976).

Early clinical signs. Sudden unexplained tachycardia, often associated with arrhythmias, is the most consistent early sign of MH. Occasionally, ventricular tachycardia or fibrillation may be the presenting sign. Blood pressure may be unstable with both hypertensive and hypotensive periods.

In spontaneously breathing patients tachypnoea occurs early, and is a consequence of the increased CO₂ production. Skeletal muscle rigidity may reduce chest wall compliance. Cyanosis and mottling of the skin may be seen. Other skin changes include sweating, flushing and vasodilatation, dryness, pallor and vasoconstriction (Britt and Kalow, 1970 a).

Skeletal muscle rigidity occurs in about 75-80% of all MH reactions (Britt and Kalow, 1970 a). The most pronounced rigidity is seen in the masseter muscle, most commonly following the administration of suxamethonium. Tracheal intubation may be difficult or even impossible. In survivors the muscles are usually oedematous and tender for several days afterwards.

In fulminant MH, body temperature may increase by 1°C every 5 minutes (Gronert, 1980b). Fulminant MH reaction may lead to brain death, and in survivors minor brain damage has been noted (Fletcher et al., 1982).

Late signs. Cardiac arrest, left ventricular failure, pulmonary oedema, renal failure, intravascular coagulation and a temperature of 43°C or more are signs associated with a poor prognosis.

Metabolic changes and laboratory findings. The increased metabolism leads to metabolic and respiratory acidosis. The integrity of the cell membrane is reduced due to insufficient supply of ATP (Britt and Kalow, 1970 b). This leads to leakage of intracellular substances such as CK, myoglobin, K, and Mg ions into the extracellular fluid. The increase in serum potassium contributes to cardiac arrhythmias. CK, which may be slightly elevated in MHS individuals at rest increases during MH to as much as several hundred times normal values. Other muscle enzymes, e.g. Aspartate-amino-transferase (ASAT) and lactate dehydrogenase (LD), are seen in increased

concentrations in serum (Rutberg et al., 1983). In porcine MH there is an increase in circulating lactate, pyruvate and alanine levels resulting from extremely rapid muscle glycogenolysis (Hall et al., 1982). A massive increase in plasma catecholamine concentrations during MH in pigs has been described (Lister, Hall and Lucke, 1974; Gronert, Milde and Theye, 1976).

Treatment of an acute MH-reaction

Symptomatic therapy. The classic advice is that administration of all known MH inducing agents should be stopped and surgery and anaesthesia should be terminated as soon as possible. However, there are several instances in Sweden of a mild acute MH being rapidly diagnosed and treated, anaesthesia thereafter being continued with intravenous agents. Cooling is necessary if the temperature is raised. Iced intravenous fluids, gastric or peritoneal lavage, and surface cooling with the patient packed in ice are usually effective in lowering the temperature. Cooling should be stopped in time to avoid hypothermia. Sodium bicarbonate should be given. Ventilation should be increased two- to threefold with 100% oxygen, using fresh tubings.

It is important to maintain a high urinary output (to prevent precipitation of myoglobin in the renal tubules) using volume loading and diuretics. The bladder should be catheterized. Insulin and glucose is recommended for treatment of hyperkalemia (Pollock and Britt, 1983). If the arrhythmia persists procaine or procainamide may be required. The drugs should be infused under continuous ECG control (Pollock and Britt, 1983).

Dantrolene. Dantrolene, a hydantoin derivative, was first described in the treatment of MH by Harrison (1975). Dantrolene was at that time already in

use for the treatment of spasticity (Dykes, 1975). The recommended dose of intravenous administered dantrolene is $1 \text{ mg kg}^{-1} \text{ min}^{-1}$ until the symptoms begin to decline. A maximum of 10 mg kg^{-1} may be given over a 15 minute period, and may be repeated every 15 minutes, if the reaction recurs (Britt, 1984). Usually a loading dose of $2.5 - 3 \text{ mg kg}^{-1}$ is enough to reverse the symptoms (Kolb et al., 1982). Britt also recommends that the initial dose of dantrolene should be followed by a maintenance infusion of $1-2 \text{ mg kg}^{-1}$ during each three to four hour period until all evidence of an MH reaction has disappeared. The intravenous dantrolene should, however, be replaced as soon as possible with oral dantrolene, to avoid peripheral thrombophlebitis. It is important to start dantrolene treatment as soon as possible, while there is still adequate muscle perfusion.

Side effects of dantrolene. Acute intravenously administered dantrolene has no severe side effects at recommended doses except for thrombophlebitis mentioned above. However, at high doses (10 mg kg^{-1}) an increase in blood pressure and systemic vascular resistance, and a decreased cardiac index have been observed in dogs (Durbin, Fisher and Lynch, 1983). Flewellen et al. (1983) describe subjective discomfort in awake humans receiving intravenous dantrolene such as dizziness, weakness, bilateral ptosis and difficulty in swallowing. All symptoms disappeared within 48 hours. Dantrolene may potentiate the effect of non-depolarizing relaxants during anaesthesia.

Anaesthesia for susceptible patients

It is important to avoid anxiety and stress to the patient, who should be well premedicated. Minimal requirements for monitoring include continuous

temperature and ECG registration which should continue for 24 hours post-operatively. Monitoring carbon dioxide elimination during anaesthesia is to be strongly recommended (Fletcher et al., 1981; Baudendiestel et al., 1985). Adequate levels of general anaesthesia should be used if this form is chosen as light anaesthesia per se may trigger an MH reaction, and many of the signs associated with light anaesthesia, such as tachycardia and sweating, are also typical of MH. Safe anaesthetics include: barbiturates, opiates, droperidol, alcuronium and nitrous oxide. Pancuronium has been suggested to be safe (Ellis and Halsall, 1980) but because of its sympathomimetic effects should perhaps be regarded with caution (Fletcher et al., 1981). If the patient is very anxious in spite of premedication, dantrolene can be used prophylactically in an intravenous dose of 2.4 mg kg^{-1} (Flewellen et al., 1983).

Pathophysiology

In order to discuss the pathophysiology of MH the mechanism of a normal contraction must first be described. Activation of the contractile system is initiated by depolarization of the sarcolemma (SL). This depolarization is then propagated via the tubular system (T-system) into the myoplasm, by a release of calcium ions from the sarcoplasmic reticulum (SR). The T-system is the functional and anatomical connection between the SL and the interior of the muscle cell. A contracture is initiated when the myoplasmic calcium concentration reaches a minimum level of around $5 \text{ } \mu\text{mol l}^{-1}$. Relaxation is possible because of an active reuptake of calcium ions by the SR, SL and mitochondria, lowering sarcoplasmic Ca^{2+} to a resting level of $<10^{-7} \text{ mol l}^{-1}$ (Walsh, 1983).

A small increase in the myoplasmic calcium level triggers release of

greater amounts of calcium ions from the SR; this has been termed "calcium induced calcium release" (Endo, Tanaka, and Ogawa, 1970). This mechanism does not seem to play an important role in physiological excitation-contraction (E-C) coupling mechanism, but is considered to be responsible for contractures induced by caffeine (Endo 1975) or halothane in MHS individuals (Takagi et al., 1976).

Most of the work on acute MH pathophysiology has been done on MH susceptible pigs or on isolated human skeletal muscles or organelles. In spite of these studies the aetiology of MH is still not known. It is, however, shown that an increased myoplasmic influx of calcium occurs during an MH reaction. This increased calcium probably initiates the muscular rigidity, metabolic disturbances and heat production. The increase in calcium ions in the myoplasm could be produced by any of the following mechanisms, alone or in combination:

1. An increased release of calcium ions from the calcium storing organelles in the muscle cell, i.e. SR, T-system and mitochondria.
2. A decreased reuptake of calcium ions from the myoplasm.
3. An increased entry of calcium ions into the myoplasm from the extracellular fluid (Denborough, 1980).

Moulds and Denborough (1974) have shown enhanced contractures induced by halothane in MHS muscles in the absence of extracellular calcium ions and conclude that an increased entry of calcium ions into the myoplasm from the extracellular fluid cannot be the only explanation for an MH reaction. However, they also showed that extracellular calcium is important in maintaining a drug induced enhanced contracture in MHS muscles, which implies a tight coupling between the release of calcium ions stored in the intra-

cellular membranes and their replacement by extracellular calcium. Investigation of the reuptake of calcium ions by the SR has given conflicting results. Kalow et al. (1970) found decreased reuptake of calcium by the SR in MHS individuals after exposure to halothane, but no difference without halothane exposure. However, both Steward and Thomas (1973) and Berman and Kench (1973) found increased uptake of Ca^{2+} in the SR of MHS pigs, although halothane did not modify this effect. Recently Blanck et al. (1981) did not find any differences between MHS and normal individuals in Ca^{2+} uptake and release function of the SR.

Endo et al. (1983) have shown that the calcium induced calcium mechanism was more sensitive to calcium in an MHS muscle than in normal muscles, and that the maximum rate of calcium release was higher. They conclude that in MHS individuals, sensitivity to the contracture-inducing action of halothane and caffeine must be due to a more labile calcium induced calcium release mechanism. Cheah and Cheah (1976) found an increased rate of mitochondrial calcium efflux in muscle of MHS pigs.

Okumura et al. (1980) have shown that the SL and the T-system are both essential for the development of a drug induced contracture in MHS swine. These results suggest that in MHS there may be abnormalities in both the SL and the T-system as well as in the SR and the mitochondria.

A common explanation for all these suggested mechanisms is an abnormal cyclic AMP (cAMP) metabolism. Stanec and Stefano (1984) recently found higher cAMP levels during exercise in MHS individuals compared to controls.

The action of dantrolene. Although the exact mechanism of action of

dantrolene is not yet known, it is believed that dantrolene inhibits excitation-contraction coupling in the muscle by inhibiting release of calcium ions from the sarcoplasmic reticulum (SR), or by directly or indirectly increasing calcium uptake into the SR (Britt, 1984). This indirect effect might be on the transverse tubules, on E-C coupling at the junctional gap between the transverse tubules and the SR, or on the mitochondria. It has often been claimed that dantrolene acts specifically on skeletal muscle (Ellis et al., 1973). Others, however, have shown that dantrolene alters the function of both smooth muscle and cardiac muscle (Graves, Dretchen and Kruger, 1978; Meyler, Wesseling and Agoston, 1976), as well as depressing catecholamine release from the adrenal medulla (Cohen and Gutman, 1979). The known effects of dantrolene do not disagree with any of the above theories of the pathophysiology of MH.

Laboratory diagnosis of MHS

After the finding that some relatives of an MH proband had raised CK levels (Denborough et al., 1970a), this was used as a screening test for susceptibility (Isaacs and Barlow, 1970). However, raised CK levels proved to be unspecific, with both false positive and false negative results (Ellis et al., 1975; King and Zapf, 1972) and therefore pharmacological studies were carried out on muscle. In our own studies there were about 18% false positive and 52% false negative results (paper I & II).

The demonstration that muscle specimens from survivors of a MH crisis showed increased in vitro sensitivity to caffeine (Kalow et al., 1970) was an important step in the development of MH diagnosis. Ellis' and his co-workers (1971) showed that halothane induced in vitro contracture in

muscle from MH patients, whereas normal muscle was unaffected. Other agents have been tried e.g. potassium chloride (Moulds and Denborough, 1974) and a combination of caffeine and suxamethonium (Halsall and Ellis, 1979) but are little used at present.

In vitro contracture tests

The in vitro contracture test is a pharmacological test based on the increased sensitivity of skeletal muscle from MHS individuals to certain agents.

Caffeine contracture test. Caffeine induces contracture, i.e. a sustained increase in basal tension, as well as an increase in electrically stimulated isometric twitches of skeletal muscle, principally by releasing calcium ions into the myoplasm from calcium storing organelles (Frank, 1962). The calcium release is caused by enhancement of calcium induced calcium release mechanism. The caffeine induced contracture is counteracted by a low free calcium concentration or a high magnesium concentration (Endo, 1975). Type 1 muscle fibres are more sensitive to caffeine and contract at lower concentrations of caffeine than type 2 fibres (Brownell and Szabo, 1982). (Type 1 fibres are defined as those fibres staining lightly for ATPase at pH 9.4 and type 2 fibres as those staining darkly). Contracture responses in normal human muscles show no changes with maturation or aging (Gronert, 1980a).

Halothane contracture test. Like caffeine, halothane releases calcium from the SR by enhancing the calcium induced calcium release mechanism (Takagi et al., 1976). However, halothane usually induces no or only a small contracture of normal human skeletal muscle whereas caffeine induces con-

tractures in all muscles at high concentrations. In normal muscle halothane enhances electrical stimulated twitches, whereas in MHS muscle these twitches tend to diminish. Nelson and Denborough (1977) showed that halothane and caffeine had agonistic effects on the excitation - contraction (E-C) coupling mechanism and thus may act at separate E-C coupling sites. Halothane, at concentrations which are without effect on calcium accumulation by isolated SR, potentiates caffeine induced contractures in normal human skeletal muscle (Moulds and Denborough, 1972; Kalow et al., 1970). Dantrolene has a greater inhibitory effect on halothane stimulated isometric twitches compared to caffeine stimulated isometric twitches (Nelson and Denborough, 1977).

Influence of fibre size. Britt et al. (1980) have shown that the weight, length or diameter of the fascicles had little or no effect on the height of the caffeine or caffeine-halothane contractures. This is probably because the contractures measured are several times below the maximum contraction force of the muscle samples.

Influence of temperature. The temperature in the muscle bath is critical. Caffeine contractures were greater at 37°C compared to 22°C whereas no such difference was found in the caffeine-halothane contracture test (Britt et al., 1980). Usually no contracture can be induced by halothane at 25°C and this test must thus be performed at 37°C (Nelson et al., 1975; Sullivan and Denborough, 1981).

Influence of calcium. As calcium plays a key-role in the aetiology of MH, investigations have been performed on the influence of extracellular calcium on the in vitro muscle test. Moulds and Denborough (1974) have

shown that contractures with caffeine or halothane were produced at zero calcium concentration in the tissue bath. However, no contracture developed after a second exposure.

Other in vitro contracture tests. The potassium induced contracture in MHS muscles is believed to be caused by an increase in resting membrane potential, which in turn starts the contractile process in susceptible muscle. A concentration of 80 mmol l^{-1} of potassium chloride has been used to ensure a complete activation of the contractile process (Moulds and Denborough, 1974). A large dose (10 mg) of suxamethonium has also shown to induce contractures in MHS muscle but not in normal muscle (Moulds and Denborough, 1974). However, Halsall and Ellis (1979) were unable to demonstrate contractures in MHS muscle induced by suxamethonium alone, but found greater response in MHS muscle compared to normal muscle, with a combination of suxamethonium and caffeine. Both these in vitro tests are, however, little used today.

Other tests in the diagnosis of MHS

Measurement of depletion of adenosine triphosphate (ATP) in muscle as a diagnostic test of MHS was originally described by Harrison et al. (1969). ATP is estimated after exposure to halothane and compared to muscle not exposed to halothane. The value of this test is, however, uncertain (Britt et al., 1976; Gronert, 1979).

Platelets have been considered for diagnostic studies in MHS since they have a contractile system and organelles for intracellular calcium storage, analogous to skeletal muscle. Solomons, Tan and Aldrete (1978) found a significant reduction in ATP and adenosine diphosphate (ADP) and

a concomitant increase in adenosine monophosphate (AMP) in MHS platelets compared to controls after exposure to halothane in vitro. However, others have not been able to reproduce these results (Giger and Kaplan, 1983; Lee et al., 1981).

A method for preparing single chemically skinned skeletal muscle fibres was originally described by Wood (1978). The fibres consisted only of sarcoplasmic reticulum and myofibrils and it was thus possible to determine release of calcium from SR and contractile effect of calcium on the myofibrils. The advantage of this method is that fibres prepared from muscle can be stored in a freezer for several months. Britt et al. (1982) have studied skinned fibres tension response to caffeine in relation to MHS. They found the method more time consuming and with more false negative results compared to the caffeine/halothane contracture test, and recommend this test only as a complement to the in vitro contracture tests.

Whittaker, Spencer and Searle (1977) described an increased incidence of the recessively inherited flouride resistant plasma cholinesterase deficiency, in MHS individuals. These findings could not, however, be confirmed by others (Evans et al., 1981; Ørding, Hanel and Viby-Mogensen, 1981). By nuclear magnetic resonance (NMR) it is possible to detect levels of high energy phosphates in muscle. A difference in ^{31}P levels between MHS and control swine after halothane and suxamethonium administration was described by Roberts et al. (1983).

THE PRESENT INVESTIGATION

My interest in malignant hyperthermia (MH) dates from 1980, when I was for the first time confronted with a patient developing an MH reaction (Fletcher et al., 1981). We were able to confirm the diagnosis of MH susceptibility by in vitro testing, according to Ellis, Harriman et al. (1978). The test was performed at the institute of pharmacology in Lund by Dr Stefan Leander. Shortly afterwards a second MH reaction occurred, and a few from other hospitals came to my knowledge. These cases were the spur to the formation of an MH investigation unit in 1981.

PATIENTS AND METHODS

A total of 215 patients from 84 families were investigated in the various studies. The patients were investigated if they had exhibited signs associated with MH (i.e. probands) ($n = 41$). The parents were investigated if the proband had not reached puberty. Relatives of MH susceptible (MHS) persons were then offered investigation. Clinical examination was performed with special attention to signs of neuromuscular disease known to be associated with MH (King, Denborough and Zapf, 1972; Pollock and Britt, 1983). 36 patients were also examined by a neurologist (paper V), who performed a more detailed examination which included manual testing of muscle strength. The result of this examination was expressed as normal or abnormal.

From the first 85 patients (paper I) blood was taken for determination of haemoglobin concentration, and the serum concentrations of sodium, potassium, calcium, magnesium and inorganic phosphate. CK concentration was

measured before muscle biopsy in 139 patients. In 86 patients CK was also measured after muscle biopsy. An ECG was taken if clinically indicated. 155 patients (paper V) received a questionnaire concerning previous and present diseases or symptoms. The patients were asked to specify positive symptoms.

Biopsy procedure

MH susceptibility was diagnosed by in vitro testing of muscle obtained from a motor point biopsy of the vastus medialis muscle. Biopsies were taken under femoral nerve blockade (n=40) with 2% procaine, 2% mepivacaine or 0.25% bupivacaine, epidural anaesthesia (n=136) with 2% mepivacaine, spinal anaesthesia (n=6) with 2% tetracaine or under thiopentone, fentanyl and nitrous oxide in oxygen anaesthesia (n=33). During anaesthesia, the ECG and rectal temperature were monitored continuously and arterial pressure was measured intermittently. Patients found to be normal on in vitro testing were observed following the procedure for about 6 hours; those found to be MHS were followed for at least 24 hours.

The biopsy specimens for the in vitro test were transported and stored in Krebs solution at room temperature. The composition of Krebs solution is shown in table I. The first 85 patients were performed using Krebs solution A, and are presented in paper I. 35 of these patients are also presented in paper IV and 84 in paper V. In 1983 the European malignant hyperpyrexia group was formed and according to a common in vitro test protocol formulated by the group (1984), the Krebs solution was changed to solution B as seen in table I. The new protocol implied some small changes in the in vitro test procedure. The two methods will be described later in

this chapter and are referred to as method A (the first 85 patients (paper I) and method B (the next 130 patients, presented in paper II, 112 in paper III, five in paper IV and 71 in paper V).

Table I. Composition of the two different Krebs solutions used in method A and B respectively. The values are expressed in mmol l^{-1} .

	<u>Krebs solution A</u>	<u>Krebs solution B</u>
Na	143	143
K	4.6	4.6
Ca	2.5	2.5
Mg	1.8	0.8
Phosphate	1.2	1.2
Glucose	5.5	11.1

In vitro test procedure

For the in vitro test, pieces of muscle 10-25 mm long and about 2-3 mm in diameter were dissected. Each of these specimens was then tied at each end with fine synthetic sutures and suspended in a tissue bath (volume 4 ml) perfused with 1.5 ml min^{-1} of Krebs solution at 37°C and bubbled with preheated 5% carbon dioxide in oxygen. The viability of the muscle specimen was demonstrated by supramaximal electrical stimulation at 0.2 Hz. The specimens were exposed to either halothane or caffeine in increasing concentrations and the muscle tension measured by a displacement transducer (Grass FT03) connected to the upper part of the muscle specimen, and a

record obtained. The lower part of the muscle specimen was attached to a small hook which in turn was connected to an eccentric wheel turned by a motor (fig. 1).

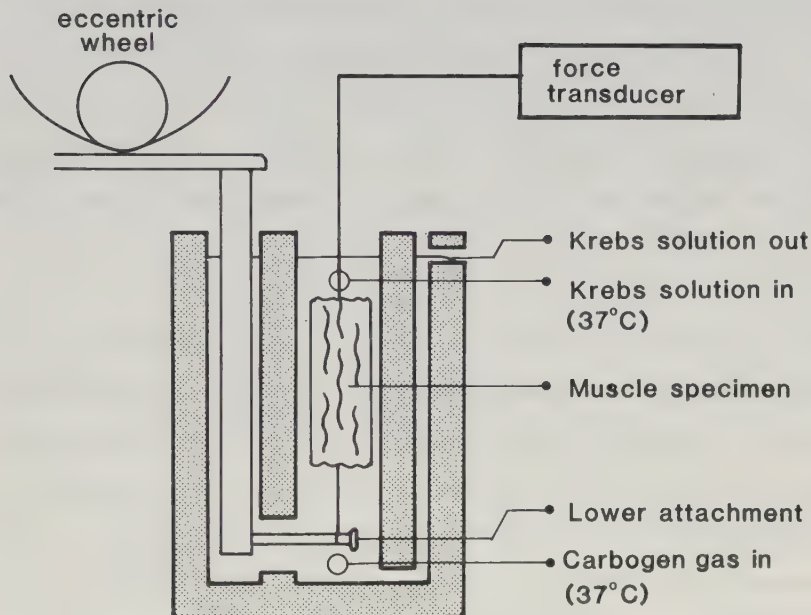


Fig. 1. The muscle bath for the in vitro test.

Two different tests, one static and one dynamic, were performed with both halothane and caffeine, making four tests in all. In the static test the muscle specimen was stretched initially to about 2 g and left for 10-20 minutes before adding halothane or caffeine. In the dynamic test the muscle was first allowed to rest at zero or low tension for three minutes and was then stretched at a rate of 4 mm min^{-1} for 1.5 minutes to a tension of approximately 2 g, left for 1 minute and then shortened at the same rate to the initial length. The whole cycle was thus 7 minutes

(Ellis, Harriman et al., 1978). After three such cycles halothane or caffeine was added.

The caffeine used was 99% pure caffeineanhydride (European Pharmacopia), with a pKa of 14. It was dissolved in Krebs solution immediately before the test was performed to achieve a stock solution of 64 mmol l^{-1} . This stock solution was then added to different containers to the final concentrations seen in table II. The concentration of caffeine at 2 mmol l^{-1} was regularly checked using high performance gas chromatography (HPLC) and was between $1.85\text{--}2.01 \text{ mmol l}^{-1}$ (mean 1.97) in the tissue bath.

Halothane was added to the carbogen gas via a Dräger vaporizer, the performance of which was regularly checked with a gas analyzer ("Emma", Engström Ltd, Sweden), calibrated against a test gas. The concentration of halothane in method A and B are seen in table II. The concentration of dissolved halothane in Krebs solution was checked using a flame ionization gas chromatography, performed by Dr Hans Sonander. He used a technique of direct injection of the halothane-Krebs solution with internal standard solutions. The coefficient of variation of the analyses was 5-7%. The concentration of dissolved halothane in Krebs solution is shown in table III. The values are about 70-80% of values expected using a partition coefficient of 0.78 (Halliday et al., 1977). These values are in agreement with those of other members of the European Malignant Hyperpyrexia Group, and were later adopted as a "target" value for the halothane test.

Table II. Concentrations of halothane (gas phase) and caffeine in Method A and B respectively.

Halothane conc (%)		Caffeine conc (mmol l ⁻¹)	
<u>Method A</u>	<u>Method B</u>	<u>Method A</u>	<u>Method B</u>
0.5	0.5	1.0	0.5
1	1	2.0	1.0
2	2	4.0	1.5
4	3	8.0	2.0
		16.0	3.0
			4.0

Table III. Concentrations of dissolved halothane in Krebs solution at different nominal concentrations of halothane.

Nominal halothane concentration (%)	Concentration of dissolved halothane (mmol l ⁻¹)	
	<u>Found</u>	<u>Expected</u>
0.5	0.11	0.14
1	0.22	0.28
2	0.44	0.57
3	0.66	0.85

In the static test the muscle was exposed to each concentration of caffeine or halothane until a plateau contracture was induced or 3 minutes had elapsed. In the dynamic test exposure was at one cycle for each concentration. In the static test a contracture was measured as an increase

in tension compared to the lowest tension obtained. In the dynamic test, the increase in tension was measured at the peak level in method A and at the plateau level in method B (fig. 2).

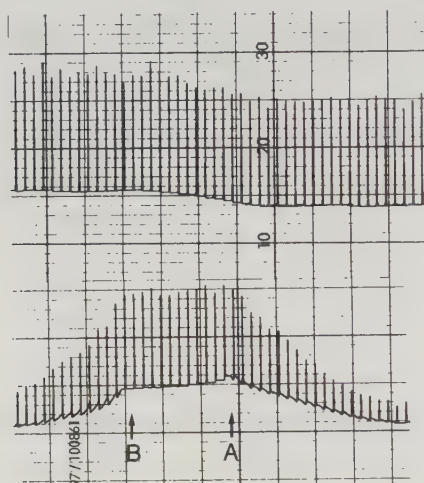


Fig. 2. Tracings from the static (above) and dynamic (below) tests. The points of measurements in the dynamic test in method A and B are indicated by arrows. The tests run from right to left.

The temperature in the tissue bath was $36.7\text{--}37.3^{\circ}\text{C}$ at a constant flow of about 1.5 ml min^{-1} of Krebs solution. During maximum flow (i.e. when the caffeine concentration was increased) the temperature rapidly rose to $38.4\text{--}39.2^{\circ}\text{C}$, but returned quickly to the initial temperature after re-establishment of the basal flow rate. Thus the muscle was exposed to an increased temperature only for a very short time.

The composition of Krebs solution A and B (table I) were regularly controlled at the chemical laboratory in the hospital using their standard procedures. pH in the tissue bath was regularly controlled using a Digit

501 pH-meter (Crison, Scandimetric). During continuous bubbling with 5% CO₂ in oxygen, pH varied between 7.32-7.56. Ionized calcium was measured using a calcium electrode (Radiometer F 2002, Bergman & Beving, Sweden) and values of $2.5 \pm 0.1 \text{ mmol l}^{-1}$ was found, e.g. all calcium in the Krebs solution was ionized. No change in ionized calcium was seen during the whole in vitro test procedure and no difference between Krebs solution and Krebs-caffeine solution was found.

Control biopsies. Criteria for in vitro diagnosis were established from control biopsies taken with informed consent from healthy subjects during minor surgery on the leg. The control patients (12 in paper I and 13 in paper II) were anaesthetized in the same way as the MH patients and the biopsies were all taken from the vastus medialis muscle. None of the control patients had experienced any anaesthetic complication, nor was there any family history of neuromuscular disease.

Comparison between static and dynamic in vitro contracture tests (paper III)

One hundred and twelve patients were investigated according to method B. Static and dynamic halothane and caffeine tests were compared respectively. The order in which the tests were performed was randomized to exclude the effects of time elapsed since removal of the specimen.

Microcalorimetric studies (paper IV)

Muscle preparation. A muscle specimen was taken for microcalorimetric studies at the same time as the biopsy for the in vitro test (method A) in

26 patients. After excision the muscle was stored and transported at 4°C in Krebs Ringer phosphate (KRP) buffer solution containing glucose 8.25 mmol l⁻¹ and insulin 0.1 IU ml⁻¹. The fibres were dissected to about 1 mm diameter (50 mg weight) and placed in the calorimeter within 30 minutes of excision. The studies were performed in a perfusion calorimeter (Wadsö, 1974) in which the fluid was maintained at 37°C by a heat exchanger to ensure minimal heat loss from the system (fig. 3). Heat production was measured as the change in voltage produced in a thermopile placed around the calorimeter vessel. After 30 minutes the perfusion fluid was changed to one equilibrated with 4% halothane.

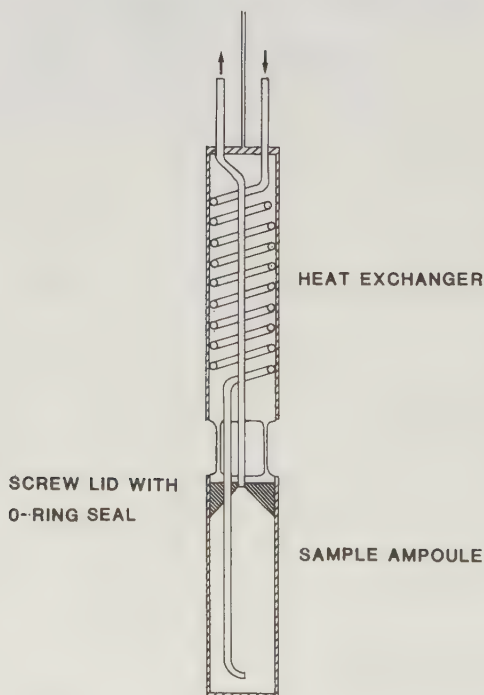


Fig. 3. Perfusion calorimeter for the microcalorimetric studies on muscle.

Platelet suspensions. Venous blood samples were collected from 14 patients under investigation for MH susceptibility, in 10 ml Vacutainer tubes containing 25 U.S.P. units of sodium heparin (Kabi, Sweden) and 2 ml of 5% Dextran solution (Pharmacia, Sweden). Platelet rich plasma (PRP) was prepared as described by Levin (1971). The concentration of platelets in the cell suspensions was about $150 \times 10^9 \text{ l}^{-1}$. Duplicate platelet and leucocyte counts were made by phase contrast microscopy. No erythrocytes were observed in the cell suspensions used. The platelet studies were performed in a microcalorimeter of the thermopile heat conduction type (Spink and Wadsö, 1976). The samples were enclosed in 1.0 ml stainless steel ampoules. Measurements were made under static conditions at 37°C as described by Monti and Wadsö (1977). Two samples were measured simultaneously. Corrections were made for the heat production of leucocytes in the PRP suspensions (2% of the total heat production (Bandmann, Monti and Wadsö, 1975)) and for the final pH to pH 7.4 (Monti and Wadsö, 1977). The coefficient of variation for the method was 4 % (Monti and Wadsö, 1977).

The effect of caffeine on platelets was studied in nine patients, (five MHS and four MH negative (MHN)) investigated according to method A. Caffeine was diluted in KRP-buffer and was then added to PRP to a final concentration of 4 mmol l^{-1} . Control ampoules contained PRP and KRP buffer solution.

The effect of halothane on platelet heat production was studied in five patients investigated according to method B (one MHS, two MHN and two MH equivocal (MHE) patients (page 34). Platelets were suspended in autologous plasma and the reactant ampoule was equilibrated with 3% halothane for 10 minutes before measurements were begun.

Histopathological and histochemical investigation (paper V)

Muscle biopsies were taken for histopathological and histochemical investigation in 84 patients investigated according to method A and 71 patients investigated according to method B. The patients' ages ranged from 13 to 66 years. Muscle pieces about 10x3x3 mm were placed immediately after excision in 4% neutral buffered formalin for histopathological investigation. For histochemical investigation similar sized muscle pieces were frozen in a mixture of iso-pentane (BDH Chemicals Ltd, Poole, G.B.) and dry ice immediately after excision. The frozen pieces were then stored in a refrigerator at -70°C until analyzed. Histopathological examination of 155 muscle biopsies was performed and 150 biopsies were examined with the histochemical method. Paraffin-embedded material was stained with hematoxylin and eosin, van Gieson, and Ladewig staining. Frozen material was stained for the following enzymes: myofibrillar adenosine triphosphatase (ATP-ase) with preincubation at pH 9.4 and pH 4.6. NADH-diaphorase (NAD: nicotinamide-adenine-dinucleotide) (Dubowitz and Brooke, 1973), phosphorylase (Eränkö and Palkama, 1961) and acid phosphatase. Periodic acid Schiff (PAS) reaction was done for glycogen. Oil-red-O staining was used for lipids. Hematoxylin-eosin, Weigert HTX-van Gieson and modified Gomori trichrome staining (Engel and Cunningham, 1963) were also done on the frozen material.

The areas of type 1 and type 2 fibres were calculated using a digitizer (Bausch and Lomb, Huston Instrument) connected to a computer. Determination of the frequency of the different fibre types and measurements of the fibre area was performed in nine MHS patients and in ten MHN patients. These biopsies were randomly selected from the whole material. The measurements were made in the area of the biopsy where the best cross-sections of fibres were found. All fibres were measured in the selected area.

Statistics

For statistical comparison between groups a Mann-Whitney test was employed. For statistical comparison in each group a Student's t-test was employed; for replies to the questionnaire a Fisher's exact test was used. The MHS and MHE group was pooled in order to compare our results with others not applying the criteria of the European MH group. Values of p less than 0.05 were considered significant.

RESULTS

Diagnostic criteria

Figures 4-7 show the results from the control biopsies in method A and B respectively (paper I and II). From these the diagnostic criteria were established. The criteria for method A are shown in table IV. None of the control muscles demonstrated a contracture above 0.2 g when exposed to 2 % or less halothane or 2 mmol l⁻¹ or less caffeine. The patients were considered to be MH susceptible (MHS) if any test fulfilled the criteria below. The criteria for method B are shown in table V and are in agreement with the European Malignant Hyperpyrexia Group (1985), except that the dynamic caffeine test also was taken in account. The test giving the greatest increase in tension, static or dynamic, was used to make the diagnosis.

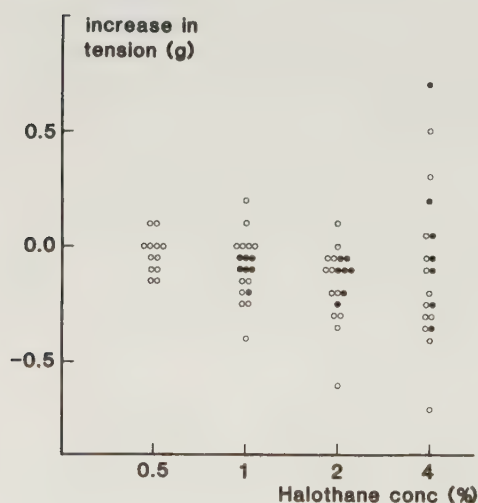


Fig. 4. Cumulative contracture response of control muscle in method A to static (●) and dynamic (○) halothane tests.

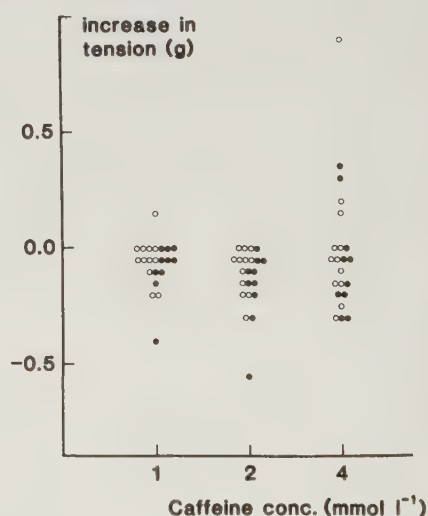


Fig. 5. Cumulative contracture response of control muscle in method A to static (●) and dynamic (○) caffeine tests.

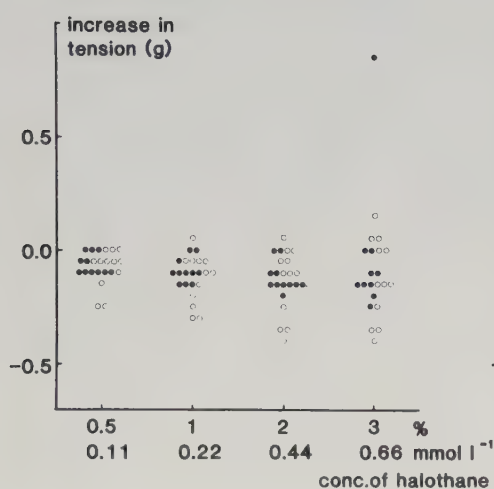


Fig. 6. Cumulative contracture response of control muscle in method B to static (●) and dynamic (○) halothane tests.

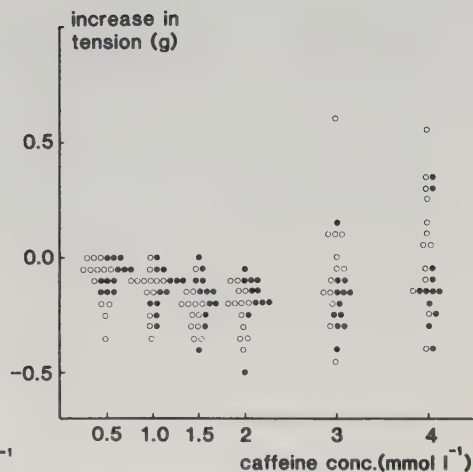


Fig. 7. Cumulative contracture response of control muscle in method B to static (●) and dynamic (○) caffeine tests.

Table IV. Diagnostic criteria for MH susceptibility in method A

Any of the following:

Static halothane test: any sustained contracture with 0.5-2%

Dynamic halothane test: a contracture above 0.2 g with 0.5-1% or a contracture above 0.3 g at 2%

Static and dynamic

caffeine test:

a contracture above 0.05 g with 1 mmol l^{-1} or

a contracture above 0.15 g with 2 mmol l^{-1}

Table V. Diagnostic criteria in method B.

<u>MHS (susceptible)</u> :	A threshold (i.e. an increase in tension of 0.2 g or more) with 0.5-2 % halothane <u>and</u> a threshold with 0.5-2 mmol l ⁻¹ caffeine.
<u>MHN (negative)</u> :	No threshold with 2 % or less halothane <u>and</u> no threshold with 2 mmol l ⁻¹ or less caffeine.
<u>MHE (equivocal)</u> :	Any other result (i.e. a threshold with <u>either</u> halothane <u>or</u> caffeine).

The main differences between criteria for method A and criteria for method B is the addition of an equivocal group (MHE) in method B, which in method A usually would have been regarded as MH susceptible. MHS is diagnosed in method B only when both the caffeine and the halothane tests are pathological.



Fig. 8. The geographical distribution of
MHS and MHE individuals in Sweden.

All patients. i.e. both those patients investigated according to method A (paper I) and those investigated according to method B (paper II) are included in the clinical evaluation. The geographical distribution of MHS and MHE patients is shown in fig. 8. Reasons for investigation and results of the in vitro test of the patients are shown in table VI and VII. The in vitro test of two patients were inconclusive and are not included in the figures. Not all the probands were biopsied; some died during MH crises and some were too young. The results from the latter have been deduced from the parents' in vitro test. 18 of 48 positive probands were referred after fulminant MH. 10 of 23 probands referred because of masseter spasm were found to be MH positive. Around 50% of relatives of a positive proband were MH positive. The types of operation during which MH occurred or was suspected are shown in table VIII. The incidence of acute operations was about the same in both the positive and negative groups.

Table VI. Type of reactions in probands from the investigated families. Number of probands biopsied in parentheses. Pooled results from paper I and paper II. * = Two or more of the following: tachycardia, arrhythmia, hypertension, rigidity, acidosis, increase in CK, myoglobinuria, fever. + = Central core disease, unexplained deaths unrelated to anaesthesia.

Type of reaction	MHS	MHE	MHN
Fulminant reaction	16 (5)	2	0
Masseter spasm	6 (4)	4 (2)	13 (8)
Possible MH reaction*	10 (3)	7 (1)	19 (13)
Unexplained fever during or after anaesthesia	0	1 (1)	3 (2)
Others ⁺	2 (1)	0	1 (1)
Total	34 (13)	14 (4)	36 (24)

Table VII. Results of in vitro tests of relatives to MHS or MHE probands.
Members of MHE families in parentheses. Pooled results from
paper I and paper II.

	MHS	MHE	MHN
Member of a family with fulminant MH	25	4 (2)	34 (3)
Member of a family with possible MH	27	4 (10)	33 (3)
Others	1	0	1
Total	53	8 (12)	68 (6)

Table VIII. Type of operation during which MH occurred or was suspected
in the proband. Number of acute operations in parentheses.

Type of operation	MHS	MHE	MHN
Abdominal	11 (8)	0	6 (6)
Caesarean section	4 (3)	0	12 (7)
ENT surgery	8 (0)	7 (1)	11 (1)
Surgery for trauma	3 (1)	4 (3)	2 (1)
Eye surgery	2 (0)	0	0
Others	4 (0)	3 (0)	4 (0)
Total	32 (12)	14 (4)	35 (15)

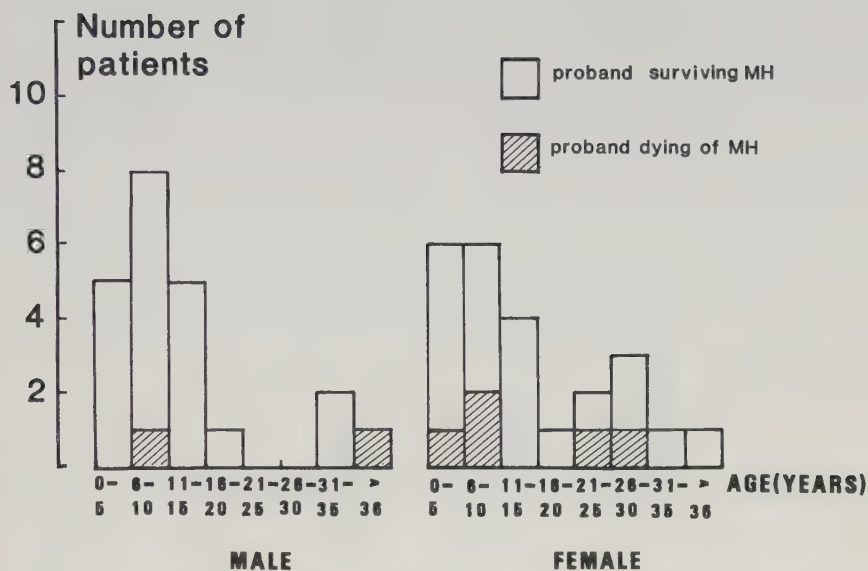


Fig. 9. Age and sex distribution of MHS and MHE probands at the MH reaction.

The age distribution and sex of the MHS and MHE probands are shown in fig. 9. Seven patients died from MH, six of these during acute surgery. Thus the overall mortality rate in this material was 15%. The mortality rate in fulminant MH was about 40%. The preoperative CK is shown in fig. 10. There is considerable spread of values, and significant difference ($p=0.001$) between MHS and MHN patients. There was no significant difference between preoperative and postoperative CK values in any group.

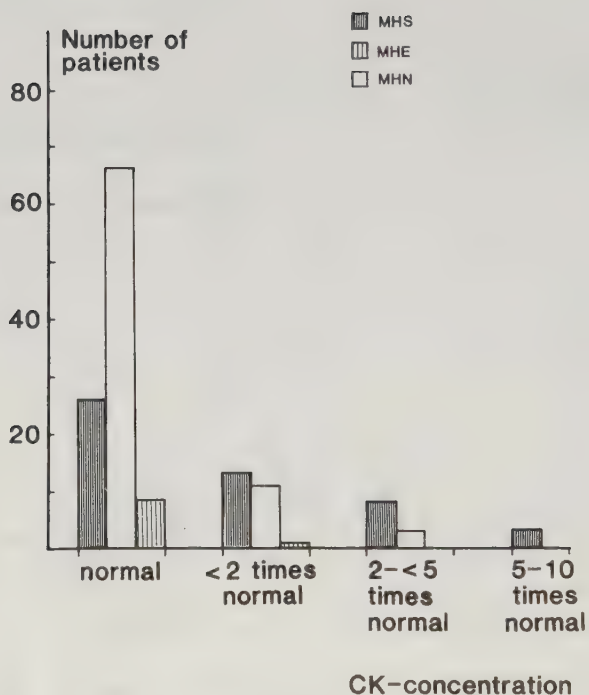


Fig. 10 Preoperative serum creatine phosphokinase (CK) concentrations.

Before the biopsy all patients had a normal temperature. Hb concentration, and the serum concentrations of Na^+ and K^+ were within normal limits. A few patients had Ca^{2+} , Mg^{2+} or inorganic phosphate values just outside the normal limits but there were no significant differences in values between MHS and MHN patients (paper I).

Forty-three patients (47%) reacted to both in vitro tests, 35 to halothane only (39%) and 11 to caffeine only (12%). Spontaneous contractures were noted in 15 patients. With two exceptions this finding was observed in muscle which reacted positively to one or both in vitro tests. One of these exceptions was the mother of a girl who died from MH. She has one brother and one son who are MHS. Thus, on the basis of the family history and the reactive muscles, we consider her susceptible. The other was the mother of a boy who had experienced a possible MH reaction. The father of this boy had a caffeine threshold at 1.5 mmol l^{-1} i.e. MHE. As we and other members of the European MH group believe that a spontaneous contracture is a pathological phenomenon, we have classed her MHE.

Comparison between dynamic and static halothane and caffeine tests respectively (paper III)

Twenty-five patients were diagnosed MHS, 20 MHE and 67 MHN. In both the MHS group ($p=0.008$) and the MHE group ($p=0.002$) thresholds with halothane were obtained at lower concentrations in the dynamic test than in the static. Five MHS patients and all but two MHE patients had normal static halothane tests but abnormal dynamic tests. Five patients in the MHE group but none in the MHS group had normal dynamic halothane tests (fig. 11).

In the caffeine test, thresholds were obtained at significantly lower concentrations in the dynamic than in the static test in both the MHE ($p=0.004$) and MHN group ($p=0.006$). However, no significant difference could be seen between the static and dynamic caffeine tests in the MHS group ($p=0.5$) (fig. 12).

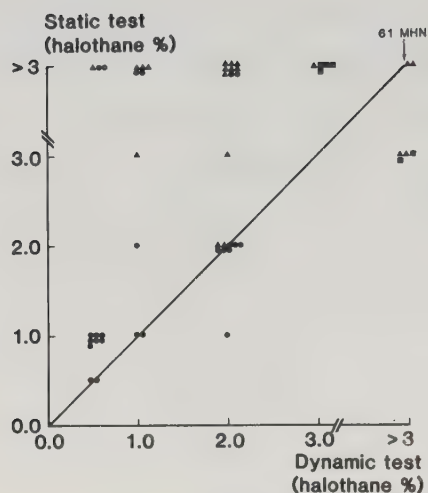


Fig. 11. Thresholds in dynamic v. static halothane tests in 25 MHS (●), 20 MHE (▲) and 67 MHN (■) patients.

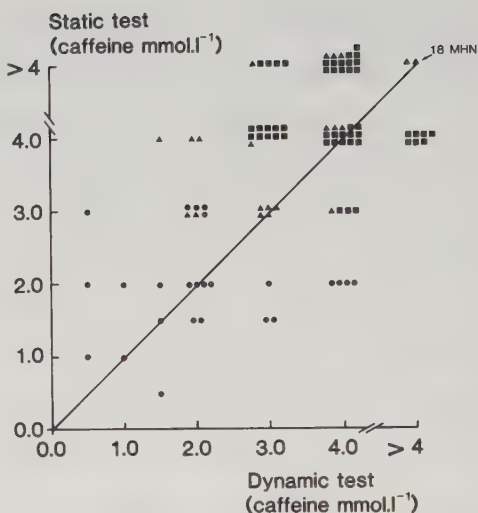


Fig.12. Thresholds in dynamic v. static caffeine tests in 25 MHS (●), 20 MHE (▲) and 67 MHN (■) patients.

Effect of halothane on heat production in muscle (paper IV)

Figure 13 shows a typical muscle calorimetry tracing. Before the addition of halothane there was a continuous decrease in heat production. The addition of halothane was in all instances followed by an increase in the rate of heat production; on many occasions the increase was enough to reverse the trend. Heat production was measured as the area under the "hump" in the tracing (the shaded area in figure 13). There were no significant differences between MHS (n=12), MHN (n=14) and normal controls (n=7).

Effect of caffeine on heat production by platelets (paper IV)

Heat production was determined with and without the addition of caffeine to a final concentration of 4 mmol l^{-1} . In all instances heat produc-

tion decreased in the suspensions containing caffeine. There was no significant difference between MHS and MHN patients.

Effect of halothane on heat production by platelets (paper IV)

In four patients (one MHS, two MHN and one MHE) halothane caused an increase in heat production; in one equivocal patient heat production decreased.

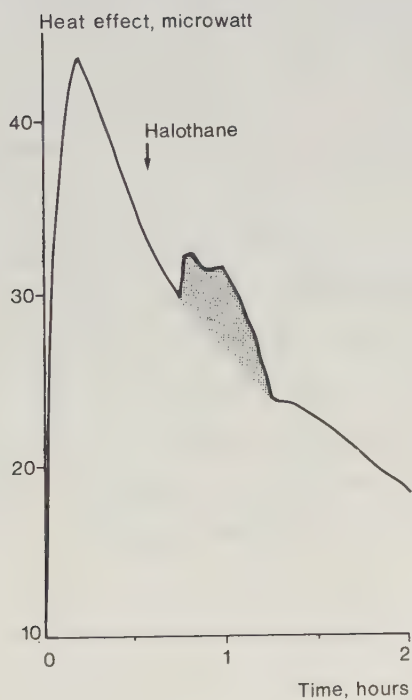


Fig. 13. Microcalorimetry tracing showing effect of halothane on muscle metabolism.

History and clinical examination (paper V)

Fifty-three patients were diagnosed MHS, ten MHE and 92 MHN. Only a minority of patients complained of any symptoms which might have been of neuromuscular origin (table IX). Amongst the MHS patients only one with central core disease (CCD) and one with myotonic dystrophy had obvious neuromuscular disease. One patient had a suprapatellar muscular atrophy and 3 patients had prominent lateral thigh muscles. The rest of the MHS patients were normal on clinical examination but one MHS proband (not biopsied), an 18 month old boy, had a severe unspecified muscular dystrophy (Fletcher et al., 1982). The sudden infant death syndrome (SIDS) was encountered in 3 families. The rest of the MHS patients were normal on clinical examination.

Amongst the MHN patients was one with muscular weakness of late onset in whom core-targetoid fibres had been found in a previous muscle biopsy, another had sarcoidosis with peripheral neuropathy causing weakness of the legs, and one a meningomyelocoele causing weakness of the legs. One MHE patient had a slight spastic tetraplegia and a Klippel-Feil deformity. 140 patients (90%) replied to the questionnaire. Amongst these patients 50 were diagnosed MHS, seven MHE and 83 MHN (Table IX). 19 control patients (76%) answered the questionnaire. There were no significant differences in the incidence of any symptoms between the groups.

Table IX. Neuromuscular symptoms reported to be associated with MH susceptibility, in MHS, MHE, MHN and control patients. Evaluation of the replies to the questionnaire: * = Frequently occurring painful muscular cramps. ⁰ = Subjective feeling of muscle weakness when climbing stairs, carrying shopping bags etc.

History or signs of	MHS	MHE	MHN	Controls
	(n=50)	(n=7)	(n=83)	(n=19)
Squint	2 (4%)	0	6 (7%)	1 (5%)
Inguinal hernia	4 (8%)	2	4 (5%)	3 (16%)
Kypho-scoliosis	2 (4%)	2	6 (7%)	0
Patellar-luxation	5 (10%)	1	3 (4%)	1 (5%)
Muscle cramps*	11 (22%)	0	13 (16%)	3 (16%)
Muscle weakness ⁰	6 (12%)	1	3 (4%)	0

Histochemical and histopathological investigation (paper V)

The findings in the muscle biopsies are presented in Table X. Stains for lipids, phosphorylase and glycogen were normal in all biopsies. As seen in the table there are no changes in the MHS group of diagnostic significance. It is, however, of interest that core-targetoid changes were found only in the MHS group, one of whom had the clinical diagnosis of CCD. The results of measurements of frequency of type 1 and type 2 fibres and of muscle fibre area are presented in Table XI and XII. There were no differences between biopsies from MHS and MHN patients in frequency of type 1 and type 2 fibres or in fibre area. The only significant difference was that the coefficient of variation of type 1 area was higher in the negative group ($p < 0.01$).

Table X. Muscle biopsy findings in MHS, MHN and MHE patients.
Some patients had more than one type of change.

Findings	MHS (n = 53)	MHN (n = 92)	MHE (n = 10)
Muscle fibre degeneration	3 (1 pat with myotonic dystrophy, 1 pat with CCD)	4 (single fibres)	-
Muscle fibre regeneration	3 (in 2 only a few fibres)	1 (in only a few fibres)	-
Inflammatory infiltrates	1	11 (in 10 the infiltrates were discrete)	-
Internally placed nuclei in a few fibres	27 (51%)	43 (47%)	7
Internally placed nuclei in several fibres	3 (1 pat with myotonic dystrophy, 1 pat with CCD)	-	-
Increase of connective tissue	2 (slight in one)	-	-
Atrophic fibres	36 (in 30 only a few fibres) (68%)	59 (in 52 only a few fibres) (64%)	7 (in 6 only a few fibres)
Uneven distribution and/or grouping of type 1 and/or type 2 fibres	1	8	-
Variation of fibre size	40 (22 more pronounced of type 1 fibres 11 more pronounced of type 2 fibres) (75%)	84 (52 more pronounced of type 1 fibres 21 more pronounced of type 2 fibres) (91%)	10 (3 more pronounced of type 1 fibres, 2 more pronounced of type 2 fibres)
Fibre type predominance	4 (predominantly type 1: one patient with CCD)	-	-
Changes in the intermyofibrillary network (e.g. moth-eaten fibres)	5 (9%)	3 (single fibres with ragged red appearance) (3%)	-
Cores	3 (one patient with CCD)	-	-
Splitting	6 (11%)	10 (11%)	1

Table XI. Frequency of type 1 and type 2 fibres in MHN patients.

M: Male
F: Female

Age	Sex	Numbers of fibres meas.	Frequency of type 1 fibres	Frequency of type 2 fibres	Area μm^2 Type 1	Area μm^2 Type 2
48	F	110	62%	38%	4009	2490
					SD 1277	1101
35	M	65	48%	52%	7030	7211
					SD 1892	1254
35	M	48	54%	46%	6868	10089
					SD 2620	2392
35	M	164	37%	63%	2811	2751
					SD 846	814
29	F	96	43%	57%	5125	3802
					SD 1366	742
25	F	98	51%	49%	3770	4490
					SD 1087	878
22	M	73	59%	41%	4754	7593
					SD 1602	882
21	F	92	51%	49%	4219	5023
					SD 1428	867
23	F	66	42%	58%	5988	6460
					SD 1882	1026
51	M	72	50%	50%	4936	7214
					SD 1164	1051
Mean 32,4 SD 10,6		Mean 88 SD 33	Mean 49,7 SD 7,7	Mean 50,3 SD 7,7	Mean 4951 SD 1356	Mean 5712 SD 2398

Table XII. Frequency of type 1 and type 2 fibres in MHS patients. M: Male
F: Female

Age	Sex	Numbers of fibres meas.	Frequency of type 1 fibres	Frequency of type 2 fibres	Area μm^2 Type 1	Area μm^2 Type 2
53	F	65	51%	49%	6704 SD 1177	5456 1276
34	M	76	41%	59%	4815 SD 750	6716 1399
36	M	91	57%	43%	5431 SD 1167	4447 811
32	M	98	46%	54%	4780 SD 804	4228 1032
32	M	78	32%	68%	5376 SD 1155	5788 1510
29	F	137	42%	58%	3576 SD 752	3270 883
19	M	119	59%	41%	3513 SD 900	4399 861
23	F	59	73%	27%	6606 SD 2297	6715 1077
18	F	90	40%	60%	4428 SD 1237	5761 852
Mean 30,7 SD 10,6		Mean 90 SD 25	Mean 49% SD 12,4	Mean 51 SD 12,4	Mean 5025 SD 1143	Mean 5198 SD 1183

DISCUSSION

In the establishment of MH susceptibility the only procedure which has stood the test of time is in vitro muscle testing. However, there was until recently a lack of agreement as to how the test should be performed or what diagnostic criteria to use. There was also a scarcity of acceptable control data. For this latter reason controls were selected from healthy individuals with no family history of neuromuscular disease. Biopsy site and type of anaesthesia were identical to those used for the diagnostic biopsies. None of the controls reacted with an increase above 0.2 g at 2% or less halothane or 2 mmol l^{-1} or less caffeine, whereas considerable variation is seen with 3 and 4% halothane and more than 2 mmol l^{-1} of caffeine (fig. 4-7). Our diagnostic criteria are therefore based on the responses to 0.5-2% halothane and $0.5\text{-}2 \text{ mmol l}^{-1}$ caffeine.

Mainly because of the choice of caffeine concentrations and differences in the composition of the Krebs solution, the European criteria have not been applied to the first 85 patients presented in paper I. They were all biopsied before the European in vitro test protocol was established.

The diagnosis MHS was made on a single positive test result in method A, whereas the European procedure at present demands positive results from both the halothane and caffeine tests (method B). However, the results obtained from the first control biopsies were sufficiently uniform to allow diagnosis to be made with a reasonable degree of confidence even in method A.

All the families (n=18) referred because of a fulminant reaction were proved to be MHS or MHE, indicating that the diagnostic criteria used both in method A and B, are reliable in the diagnosis of MH susceptibility.

In this material, 44% of those investigated because of masseter spasm as a sole sign of MH, proved to be MH positive; this is a little less than the 57% of Rosenberg and Reed (1983). 47% of those investigated because of two or more symptoms suggestive of MH was MH positive (table VI), which differs from the results of Rosenberg and Reed (1983), who found 8% of MHS in this group. This difference is probably the result of variation in the clinical history. Most of our patients in this group had a combination of symptoms suggestive of MH. This would be expected to result in a higher proportion of MHS when compared with a group such as Rosenberg and Reed's with only single symptoms such as unexplained tachycardia or rigidity. It is clear that masseter spasm alone or combined with other MH signs must be regarded as MH and appropriate treatment instigated.

Most MH reactions in this material occurred during three types of surgery: ENT (33%), abdominal (24%) and surgery for trauma (15%). The types of operation during which MH occurred probably reflect the types which are common in this age group. 75% of the patients referred because a possible MH reaction during caesarean section were MH negative. The high number of negative patients in this group may be attributed to infection secondary to early rupture of the membranes, causing signs resembling MH, such as pyrexia, tachycardia and acidosis. It is worth mentioning that anaesthesia for squint, hernia, or spinal deformity did not carry a higher likelihood of MH in this material, in contrast to others' findings (Wilson and Ellis, 1979, Ellis and Halsall, 1980). The ages of MH susceptible probands are

in accordance with others' findings (Ellis and Halsall, 1980; Britt and Kalow, 1970a). The overall mortality rate in this material was 15%. However, after 1981 there have been no deaths related to MH in Sweden, as far as is known by us.

About 50% of relatives to a positive proband were MH susceptible (table VII), confirming the theory that MHS is inherited as a single autosomal dominant gene. (Ellis and Halsall, 1980; King, Denborough and Zapf, 1972).

Forty-eight per cent of MHS patients had increased CK concentrations, an incidence similar to that reported by Ellis, Cain and Harriman (1978). However, in 18% of MH negative patients without any muscular symptoms the concentration of CK was increased. Thus, creatine kinase (CK) was of little value in diagnosing MH (fig. 10), even in families with fulminant MH.

In the author's laboratory the halothane test is the most reliable diagnostic test for MH susceptibility, since 86% of MHS and MHE patients had an abnormal response to halothane but only 59% to caffeine. 12% reacted to the caffeine test only. The findings of Rosenberg and Reed (1983) are similar.

In 15 patients spontaneous contractures were noted immediately after transfer of the muscle specimen to the muscle bath. With one exception they were undoubtedly susceptible. No spontaneous contracture was noted in any control muscle. This finding, which has also been observed by others (Ellis F.R. personal communication; Moulds R.F.W. personal communication) may be regarded as an extreme expression of muscle disease.

Our results demonstrate that the dynamic halothane test is more sensitive in discriminating between MHS and MHN patients than the static halothane test (fig. 11); if the static halothane test alone were used many MH susceptible patients could be misdiagnosed. Regardless of which diagnostic criteria are chosen within reason, the dynamic halothane test is still the more sensitive since the majority of points in fig. 11 are on or above the line of identity. The opposite was found with caffeine, where thresholds were obtained at significantly lower concentrations in the dynamic than in the static test in the MHN group, whilst there was no difference in the MHS group (fig. 12). The dynamic caffeine test is thus of less value in discriminating between MHS and MHN patients than the static caffeine test. This difference between the halothane and caffeine tests supports the suggestion that halothane and caffeine have different modes of action on muscle (Nelson and Denborough, 1977; Gallant, Godt and Gronert, 1979).

The results from the MHE group are difficult to interpret. Only four MHE patients had a pathological contracture with caffeine (and only in the dynamic test) but not with halothane. It is impossible to ascertain their true status from the history and pedigree. The other 14 MHE patients had normal responses to caffeine in both tests. Because of their pedigree at least two of these must be susceptible. Both have a pathological contracture to dynamic but not static halothane tests and would have thus been diagnosed as MHN without the use of the dynamic halothane test.

At present the European consensus of opinion, to which the author subscribes, is that both the halothane and the caffeine test are necessary to diagnose MH susceptibility. This opinion may be revised, of course, when more control data becomes available. Both static and dynamic halothane

tests are considered necessary, as our results confirm. However, nothing would appear to be gained by including a dynamic caffeine test in the protocol, and this test is no longer performed in the author's laboratory.

Measurement of heat production in cells by calorimetry gives information about total cellular metabolic activity. The present method of muscle fibre preparation has been used extensively by other investigators (Lundholm et al., 1975) who were unable to demonstrate morphological or functional alterations in the specimens (Bylund et al., 1976).

Muscle for this test was excised and tested at the same time as the muscle used for the in vitro halothane and caffeine tests, during which satisfactory viability was demonstrated by repeated electrical stimulation. In the model described above, halothane increased the metabolism of muscle tissue, but there was no significant difference between MHS and MHN individuals. The muscle specimen was not stretched during the test. In the in vitro test for MH susceptibility, a halothane contracture cannot usually be demonstrated at zero tension. It may, therefore, be necessary to stretch the muscle to 1-2 g tension in order to observe a difference between MHS and MHN individuals.

Halothane had a variable effect on heat production by platelets; increasing it in four patients and decreasing it in one. Although the MHS patient showed the greatest increase in heat production, the differences were too small to be of any diagnostic value. More importantly, halothane has a tendency to make platelets aggregate. Because aggregation per se causes an increase in heat production by platelets (Ross et al., 1973) we do not believe that this method can have any diagnostic value.

Caffeine, on the other hand, decreased heat production in both MHS and MHN platelets, although the effect of caffeine on the intact organism is to increase heat production (Acheson et al., 1980). Other xanthine derivatives have been shown to have the same effect on platelets (Monti et al., 1986). We conclude that the microcalorimetric methods described above cannot be used to distinguish between tissues from MHS and MHN individuals.

Harriman (1982) presented a series of 200 biopsies from persons who have been subjected to the *in vitro* test. All biopsies were, as in our study, taken from the vastus medialis muscle. 120 biopsies from MHN persons were examined, of which half showed some abnormalities. In 35 biopsies of 80 from MHS patients pathological changes were found. Harriman concluded that there are changes that are characteristic of MH-myopathy: increased internal nuclei, moth-eaten fibres and core-targetoid fibres, changes which are not, however, often observed in asymptomatic MHS patients. Our own study suggests, however, that with the possible exception of fibres with core-targetoid changes there are no findings that are characteristic or diagnostic of MH myopathy. In our study there were three MHS patients with core-targetoid fibres in the muscle biopsy and they are described below.

Target fibres are defined as muscle fibres with rounded areas devoid of staining for oxidative enzymes, and with three concentric zones - one with no staining, one with increased staining and one with normal staining. The fibre is targetoid when the zone with increased staining is absent or indistinct. Cores are defined as almost circular areas with no staining for oxidative enzymes (the core is sometimes surrounded by a dense rim). It is sometimes difficult to differentiate between target, targetoid and core

changes. The term core-targetoid fibres was used by Harriman (1982), in the above mentioned study, and is also used in this study. Target fibres and targetoid fibres are also found in muscle biopsies from patients with neuropathy. Patients with neuropathy having target or targetoid fibres have not to our knowledge been shown to be MHS. Core-targetoid fibres may indicate MHS only when they are found in biopsies without other signs of neuropathy.

The first patient with core-targetoid fibres had also the clinical diagnosis of central core disease. The patient was a 47 year old woman with slowly progressive muscular weakness since early childhood. She had been anaesthetized twice without developing MH symptoms, but had a positive in vitro test (fig. 14). The second MHS patient found to have core-targetoid fibres had no clinical symptoms of neuromuscular disease (fig. 15). He was the 16 year old brother of a girl who died of acute MH. The biopsy from the third patient, a 32 year old man, is of special interest. The biopsy was taken 3 months after acute MH. Core-targetoid and moth eaten fibres were found only in one part of the biopsy next to an area of severe muscle fibre destruction (fig. 16). In other parts of the biopsy only minimal changes were found, and in these areas there were no core-targetoid changes. These examples indicate that the changes in the muscle that can lead to MH also can lead to the development of core-targetoid changes and that these changes can be associated with MH regardless of whether they occur in muscle from patients with central core disease or those with no overt neuromuscular disease. It should be observed, however, that one patient in the MHN group had muscular symptoms and core-targetoid changes in a previous muscle biopsy.

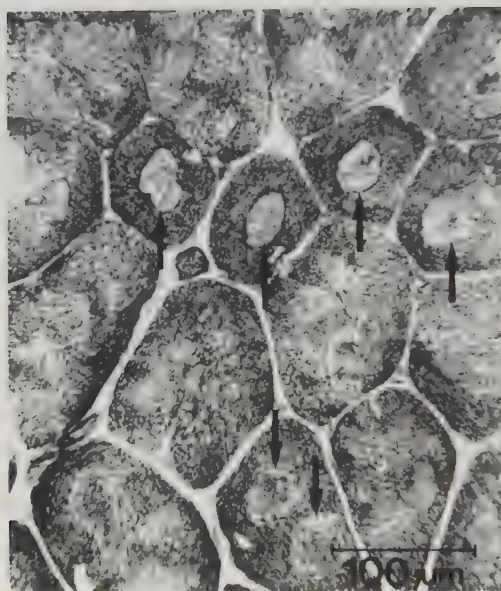


Fig. 14. Biopsy from a 47 year old woman with central core disease. Medial vastus muscle. Staining for NADH-diaphorase. Arrows show core-targetoid changes.

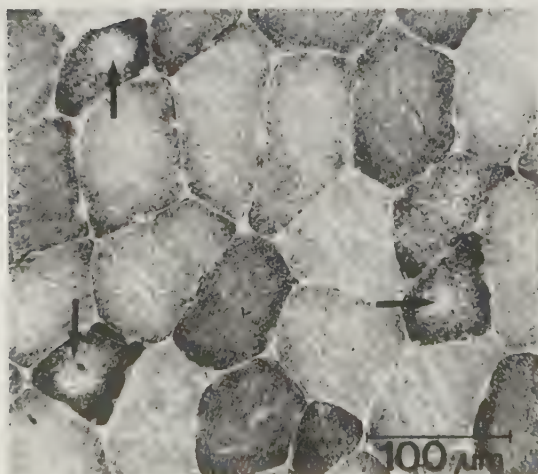
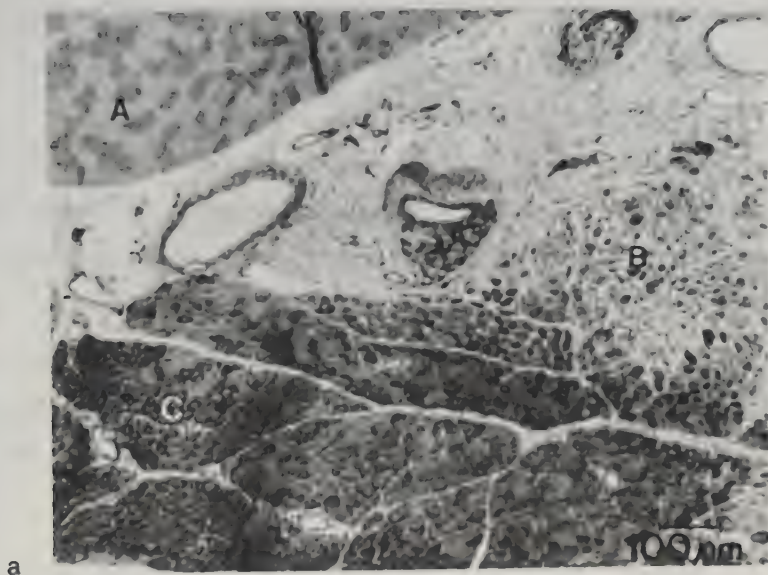
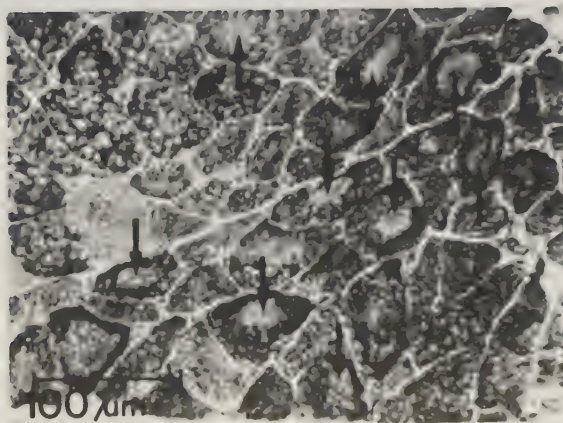


Fig. 15. Biopsy from a 16 year old boy with no neuromuscular symptoms. Medial vastus muscle. Staining for NADH-diaphorase. Arrows show core-targetoid changes.



a



b

Fig. 16. Biopsy from a 32 year old man taken 3 months after an attack of MH. Staining for NADH-diaphorase. 16a) At A normal muscle. At B fibrosis and severe destruction of muscle tissue. At C fibres with core-targetoid changes. These changes are shown at higher magnification in fig. 16 b.

The clinical investigation and the questionnaire did not show any differences between the groups in the frequency of squint, hernia, kypho-scoliosis, patellar luxation, muscular weakness or muscle cramps.

We conclude that most MHS subjects do not have a specific myopathy. They are healthy individuals with the same incidence of minor muscular symptoms and microscopic changes to normals. Our results indicate that in vitro testing for MHS should be considered if there are core-targetoid fibres in muscle biopsies, without clinical or microscopic signs of neuropathy. A histochemical and histopathological investigation could, however, not be used as a screening for MH susceptibility.

GENERAL CONCLUSIONS

The most reliable diagnostic test for MHS was the halothane test. The dynamic halothane test was more sensitive in discriminating between MHS and MHN individuals than the static. The microcalorimetric investigation of muscle and platelets was of no value in the diagnosis of MHS.

The majority of MHS patients were healthy individuals with the same incidence of clinical and subclinical myopathies as normals. Histopathological and histochemical investigation cannot be used as a screening test for MHS.

There was a wide spectrum in the severity of MH reactions in patients referred for investigation. All patients referred because of fulminant MH proved to be MHS or MHE. About 45% of those with non-fulminant signs of MH were found to be MHS or MHE.

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INVESTIGATION OF MALIGNANT HYPERTHERMIA IN DENMARK AND SWEDEN

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SUMMARY

Units for the investigation of susceptibility to malignant hyperthermia (MH) were set up in Denmark in 1977 and in Sweden in 1981. Two hundred and ten patients from 76 families have been investigated. The diagnosis of MH susceptibility (MHS) was made by *in vitro* exposure of muscle from vastus medialis to halothane and to caffeine. MHS criteria for the patients in this paper were established from examination of 31 control biopsies, obtained from the same muscle and with the same anaesthesia as the MH patients. The criteria have since been changed to those presented elsewhere in this issue. In our laboratories the halothane test (exposure to 0.5–2% halothane) was the more sensitive: 88% of MHS patients reacted to it. The caffeine test was positive in 68% of MHS patients, 0.5–2.0 mmol litre⁻¹ solutions being the most discriminating. Forty-two percent of MHS patients reacted to only one test. Fulminant MH was the most common reason for investigation; all these families contained MHS members. Masseter spasm occurred as sole sign in 21 families, of which 11 were MHS. Only 10% of MHS patients had other signs or symptoms of neuromuscular disease such as muscle cramps or muscular dystrophy. Three families had experienced sudden infant death syndrome (SIDS), and two teenage brothers in a MHS family died suddenly, but death was unrelated to anaesthesia.

Malignant hyperthermia (MH) is a rare, serious pharmacogenetic condition which manifests itself during anaesthesia (Denborough et al., 1962). Susceptibility to MH (MHS) can be determined by *in vitro* exposure of muscle to halothane (Ellis et al., 1978) or caffeine (Kalow, Britt and Richter, 1977). Units were set up in 1977 and 1981 in Denmark and Sweden, respectively, for the purpose of investigating patients and families suspected of being MHS. This paper reports the results of the first 210 investigations. In 1983 the *in vitro* test procedure was altered, following the formation of the European MH Group (The European Malignant Hyperpyrexia Group, 1984). It is therefore appropriate to review the 210 investigations performed before this change.

PATIENTS AND METHODS

Families were investigated if a member (the proband) exhibited symptoms or signs associated with MH. The proband was investigated first. If the proband had not reached puberty the parents were investigated in Sweden, whereas in Denmark the investigation was postponed until the proband reached about 10 years-of-age. Relatives of positive

probands were then offered investigation.

One hundred and twenty-five patients in Denmark and 85 patients in Sweden were investigated. Clinical examination was performed with special regard to neuromuscular signs known to be associated with MH (King, Denborough and Zapf, 1972; Pollock and Britt, 1983). Blood was drawn for determination of Hb concentration, and the serum concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺ and inorganic phosphate. Creatine kinase (CK) concentration was measured before and after muscle biopsy. An electrocardiogram (ECG) was taken routinely in Denmark, and if indicated by history or findings in Sweden.

Biopsy procedure

Susceptibility to MH was diagnosed by *in vitro* testing of muscle obtained from a motor point biopsy of the vastus medialis muscle. The biopsies were taken under thiopentone, fentanyl and nitrous oxide in oxygen anaesthesia in all the Danish patients and in 23 of those in Sweden. The rest of the Swedish patients received femoral nerve blockade ($n = 40$), extradural anaesthesia ($n = 16$) with 2% mepivacaine, or spinal anaesthesia ($n = 6$) with 2% tetracaine. During anaesthesia the ECG and rectal temperature were monitored continuously and arterial pressure was measured intermittently. Patients found to be normal on MH testing were observed following the procedure for 6–24 h, and

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those found to be MHS for at least 24 h. The biopsy specimens for the *in vitro* test were transported and stored in Krebs solution at room temperature.

In vitro test procedure

For the *in vitro* test, pieces of muscle 10–20 mm long and about 3 mm in diameter were dissected and suspended in a tissue bath perfused with Krebs solution at 37°C and bubbled with preheated 5% carbon dioxide in oxygen (carbogen). Viability of the muscle tissue was demonstrated by supramaximal electrical stimulation at 0.2 Hz. The specimens were exposed to either halothane or caffeine in increasing concentrations and the muscle tension measured by a displacement transducer, and a record obtained.

Two different halothane tests were performed, one static and one dynamic. In the static test, the specimens were stretched, initially, to a tension of approximately 2 g and left for about 10 min. After this halothane was added to the carbogen in concentrations of 0.5, 1, 2, and 4% for 3–5 min each. In the dynamic test the muscle was first allowed to rest at zero or low tension for 3 min, and was then stretched at a rate of 4 mm min⁻¹ for 1.5 min to a tension of approximately 2 g, left for 1 min and then shortened at the same rate to the initial length. The whole cycle was thus 7 min (Ellis et al., 1975). After three such cycles 0.5, 1, 2, and 4% halothane was added for one cycle each.

In both countries, a static caffeine test was performed by perfusing the muscle at a resting tension of approximately 2 g for 3–5 min with Krebs solution containing caffeine in increasing concentrations: in Denmark 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, and 16.0 mmol litre⁻¹ and in Sweden 1.0, 2.0, 4.0, 8.0, and 16.0 mmol litre⁻¹. In Sweden, a dynamic caffeine test was performed in the same way as the dynamic halothane test, with one complete cycle at each caffeine concentration. In some patients not all tests were possible because of a lack of suitable muscle.

Criteria for a positive *in vitro* test were established from control biopsies taken from healthy subjects during minor operations on the leg. The control patients (Denmark 19, Sweden 12) were anaesthetized in the same way as the MH patients. None of the controls had experienced any anaesthetic complications, nor was there any family history of neuromuscular disease. The control biopsies were all from the vastus medialis muscle.

For statistical comparison between groups a

Mann–Whitney test was used. Values of $P < 0.05$ were considered significant.

RESULTS

In Denmark 125 patients aged between 8 and 75 yr, from 44 families, were investigated with biopsy; in Sweden 85 patients aged between 13 and 64 yr, from 32 families were studied. The material also included 18 non-biopsied probands from the investigated families. No patient developed signs of MH in the perioperative period.

In vitro test

Figures 1–3 show the results from the control biopsies. From these we established the following criteria for MHS: Any sustained contracture (1) with 0.5–2% halothane in the static test; (2) above 0.2 g at 0.5–1% and above 0.3 g at 2% halothane in the dynamic test; (3) with 0.5 or 1 mmol litre⁻¹ of caffeine in Denmark, and above 0.05 g at 1 mmol litre⁻¹ or above 0.15 g at 2 mmol litre⁻¹ caffeine in Sweden. The patients were considered to be MHS if any test fulfilled the above criteria. Patients not fulfilling these criteria were deemed to be MH-negative. However, in order to accommodate to the criteria applied presently by the European Malignant Hyperpyrexia Group, our criteria have since been changed to theirs, presented elsewhere in this issue, mainly to include a group of patients with equivocal *in vitro* test results.

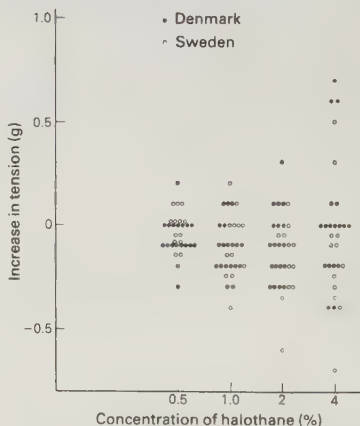


FIG. 1. Cumulative contracture response of control muscle to dynamic halothane test.

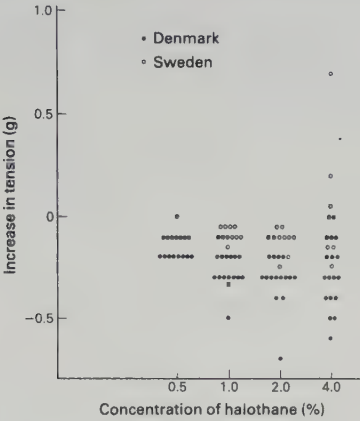


FIG. 2. Cumulative contracture response of control muscle to static halothane test.

The reasons for investigation, and the results of the *in vitro* test of the patients are shown in tables I and II and figures 4–6. The *in vitro* test of one Danish and one Swedish patient were inconclusive and are not included in the figures. Not all the probands in table I were biopsied; some died during MH crises and some were too young. The results for the latter have been deduced from the parents' *in vitro* test. The group “others” in table I includes two Swedish teenage brothers who died suddenly and unexpectedly. The cause of death could not be established at postmortem (Ranklev, Fletcher and

TABLE I. Type of reaction in probands from the investigated families. Number of probands biopsied in parentheses. *Two or more of the following: tachycardia, arrhythmia, hypertension, rigidity, acidosis, increase in CK, myoglobinuria, fever. †Central core disease, unexplained deaths unrelated to anaesthesia

Type of reaction	Positive		Negative	
	Den	Swe	Den	Swe
Fulminant MH reaction	15(4)	11(5)	0	0
Spasm of masseter muscles	7(7)	4(4)	6(6)	4(2)
Possible MH reaction*	5(5)	5(1)	4(4)	4(3)
Unexplained fever during or after anaesthesia	1(1)	0	3(3)	2(1)
Others †	0	3(1)	1(0)	0
Total	28(17)	23(11)	14(13)	10(6)

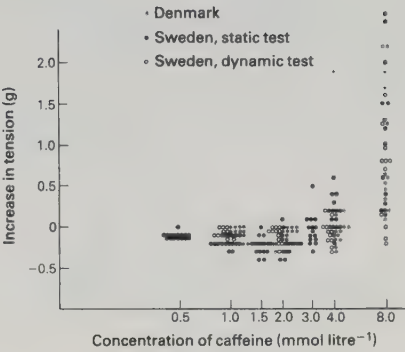


FIG. 3. Cumulative contracture response of control muscle to caffeine test.

Krantz, 1984). The mother (included in this material) had a normal *in vitro* test, whereas the father (tested later and not included here) was MH-positive.

One Danish patient died during treatment for acute self poisoning with anticholinergic and neuroleptic drugs. He was rigid and received suxamethonium to facilitate intubation without any relaxation being obtained. His temperature at this time was 40 °C. His symptoms could have been attributable to the malignant neuroleptic syndrome, to an overdose of anticholinergics or to MH. His mother had committed suicide 2 years earlier. The father and two siblings had normal *in vitro* contracture tests, and the patient was therefore considered to be MH-negative.

TABLE II. Results of *in vitro* test of relatives to MHS-probands

	Positive		Negative	
	Den	Swe	Den	Swe
Member of family with fulminant MH	40	16	32	19
Member of family with possible MH	4	12	16	19
Others	0	0	3	1
Total	44	28	51	39

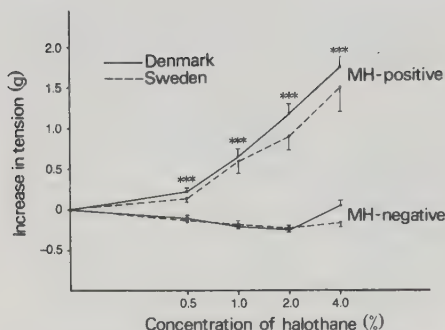


FIG. 4. Mean ($\pm$ SEM) contracture responses of diagnostic biopsy specimens in dynamic halothane test. ***Statistically significant difference between positive and negative groups ($P < 0.001$).

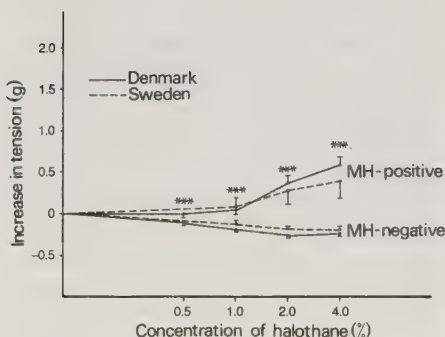


FIG. 5. Mean ($\pm$ SEM) contracture responses of diagnostic biopsy specimens in static halothane test. ***Statistically significant difference between positive and negative groups ($P < 0.001$).

TABLE III. Correlation between halothane and caffeine tests

Finding	Den	Swe
Halothane and caffeine test positive	32 (70%)	16 (42%)
Only halothane test positive	11 (24%)	15 (39%)
Only caffeine test positive	3 (7%)	6 (16%)
Spontaneous contracture only	0	1 (3%)
Total	46	38

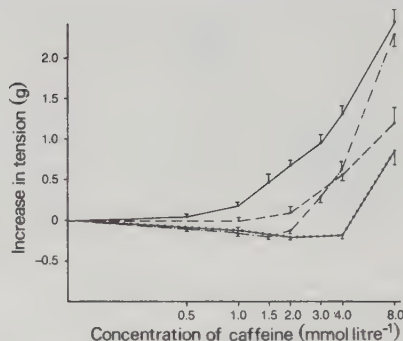


FIG. 6. Mean ($\pm$ SEM) contracture responses of diagnostic biopsy specimens in caffeine test. — Denmark, MH positive; - - - Denmark, MH negative; . . . Sweden, MH positive, static test; - . . . Sweden, MH negative, static test. $P < 0.001$ between positive and negative Danish groups for caffeine concentrations 0.5–2 mmol litre⁻¹. Significant difference between positive and negative Swedish groups for caffeine concentrations 2 and 4 mmol litre⁻¹ in static test. For the sake of clarity, the results of the dynamic test (Sweden) have been omitted.

Table III shows the relationship between the halothane and caffeine test results. Thirty-five MHS patients reacted to one of the *in vitro* tests and 48 reacted to both tests. Spontaneous contractures were noted in three Danish and 13 Swedish patients. With one exception, this finding was observed in muscle which reacted positively to one or both *in vitro* tests. The exception was the mother of a girl who died from MH. She has one brother and one son who are MH-positive. Thus, on the basis of the family history and the reactive muscles, we considered her positive.

TABLE IV. Type of operation during which MH occurred or was suspected in the proband. Pooled results from both countries. Number of acute operations in parentheses

Type of operation	Positive	Negative
Abdominal surgery	18 (12)	7 (7)
Otorhinolaryngological surgery	12 (0)	12 (1)
Surgery for trauma	10 (5)	1 (1)
Eye surgery	2 (0)	0 (0)
Others	6 (1)	3 (0)
Total	48 (18)	23 (9)

The types of operation during which MH occurred or was suspected are shown in table IV. Age distribution and sex of the MHS probands are shown in figure 7. Ten patients died from MH, eight of these during acute surgery. Thus, the overall mortality rate in this material was 21%; the mortality rate in fulminant MH was 38%.

History and clinical examination

There were no symptoms or signs of neuromuscular disease in any of the MHN patients or in 89 MHS patients. There were positive findings in 12 MHS patients. These included one with dystrophica myotonica, one who was mentally retarded and deaf, and one with atrophy of the neck muscles after arthrodesis of the cervical column (all three referred because of spasm of the masseter muscles after suxamethonium). There was also one patient with suprapatellar atrophy of the quadriceps. Five patients suffered from severe muscle cramps, and one of these experienced recurrent patellar luxation. One patient was referred because of central core disease (CCD) and one was a known carrier of Duchenne muscular dystrophy (she had two sons with this disease, one of whom survived a fulminant episode of MH). One proband, an 18-month old boy, had a severe unspecified muscular dystrophy (Fletcher et al., 1982). The sudden infant death syndrome (SIDS) was encountered in one Danish and two Swedish families.

Before surgery all patients had a normal temperature. Hb concentration, and the serum concentrations of Na^+ and K^+ were within normal limits. A few patients had Ca^{2+} , Mg^{2+} or inorganic phosphate values just outside the normal limits, but there were

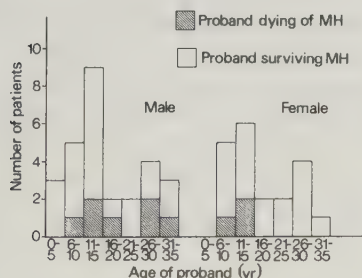


FIG 7. Age and sex distribution of positive probands. Pooled data from both countries.

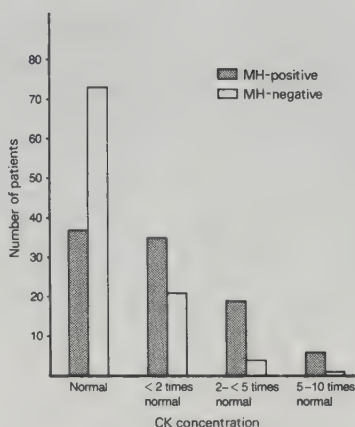


FIG 8. Preoperative serum creatine kinase concentrations. Pooled results from both countries.

no significant differences in values between MHS and MHN patients. Preoperative CK values are shown in figure 8. There is considerable spread of values, and significant difference between MHS and MHN patients.

DISCUSSION

In vitro muscle tests are used in many centres for the investigation of susceptibility to MH. However, no universal agreement exists as to which pharmacological agent best separates MHS from MHN patients or how the test should be performed. This problem is augmented by the scarcity of acceptable control data. For this reason our controls were selected from healthy individuals with no family history of neuromuscular disease. Biopsy site and type of anaesthetic were identical to those used for diagnostic biopsies. The results from the controls are fairly uniform at low concentrations of halothane and caffeine, whereas considerable variation is seen with 4% halothane and more than 2 mmol litre⁻¹ of caffeine (figs 1-3). Our diagnostic criteria for MHS are therefore based on the responses to 0.5-2% halothane and caffeine 0.5-2 mmol litre⁻¹. The nine patients investigated who had previously experienced an unequivocal MH reaction all had positive *in vitro* tests, suggesting that the above criteria are reliable in identifying MHS individuals.

Mainly because of the choice of caffeine concent-

rations and differences in the composition of the Krebs solution, we have not applied the European criteria (The European Malignant Hyperpyrexia Group, 1984) to our patients, who were all biopsied before the European "protocol" was formulated. We have been able, in our laboratories, to discern increases in tension smaller than those at present used in the European procedure. In addition, we were previously prepared to make the diagnosis MHS on a single positive test result, whereas the new procedure demands, at present, positive results from both the halothane and caffeine tests. However, the results obtained from the control biopsies were sufficiently uniform to allow diagnosis to be made with a reasonable degree of confidence.

In our hands the halothane test is the most reliable diagnostic test for MHS, since 88% of the positive patients had an abnormal response to halothane, but only 68% to caffeine. Rosenberg and Reed (1983) reached the same conclusion. However, 11% of the positive patients in our material reacted to the caffeine test only, and therefore this test has its place also.

It is apparent from figures 4 and 5 that fairly uniform results are obtained in Denmark and Sweden for the halothane test, whereas there was a marked difference in sensitivity to caffeine (fig. 6). This could be the result of different magnesium concentrations in the Krebs solution (Denmark $1.2 \text{ mmol litre}^{-1}$, Sweden $1.8 \text{ mmol litre}^{-1}$) because the calcium-releasing action of caffeine from the sarcoplasmic reticulum is antagonized by an increase in magnesium concentration (Weber, 1968). The difference between the halothane and caffeine results supports the suggestion that halothane has other modes of action as, for example, on the sarcolemma (Gallant, Godt and Gronert, 1979).

In 16 patients spontaneous contractures were noted immediately after transfer of the specimen to the muscle bath. This finding, which has also been observed by others (Ellis, F. R., personal communication; Moulds, R. F. W., personal communication) may be regarded as an extreme expression of muscle disease.

Fifty-two percent of patients investigated because of suxamethonium-induced masseter spasm were found to be MHS. This is in agreement with the 57% reported by Rosenberg and Reed (1983) for this group. However, for the group investigated because of atypical reactions suggestive of MH, our results differ from theirs: they found 8% to be MHS whereas we found 56% to be MHS. This difference is

probably the result of variation in the clinical history. First, seven of 12 patients were investigated because of unexpected fever, and in this group we found a low prevalence of MHS (one out of six patients). Second, most of our patients in this group had a combination of symptoms suggestive of MH. This would be expected to result in a higher proportion of MHS when compared with a group such as Rosenberg and Reed's with only single symptoms such as unexplained tachycardia or rigidity. Clinically, masseter spasm alone or combined with other symptoms suggestive of MH must be regarded as such and the patient treated as if MH were developing.

Most MH reactions in our material occurred during three types of surgery: abdominal (38%), otolaryngological (25%), and surgery for trauma (21%) (table IV). Twenty-one percent of MH cases reviewed by Ellis and Halsall (1980) were also seen during surgery for trauma. Recently, Pollock and Britt (1983) reviewed a large series of MH reactions, and from their data it can be calculated that approximately 25% of the cases had occurred during otorhinolaryngological surgery, —a value identical with our figure. It is interesting that in our material all surgery in this group was elective, because overall, we found a high proportion of reactions (38%) during emergency surgery. This might reflect the impact of stress on the probability of a susceptible individual developing MH. Certainly mortality was much higher during acute surgery: eight of 18 reactions *v.* two of 30 reactions during elective surgery ($P < 0.01$).

Remarkably few patients had any neuromuscular symptoms or signs known to be associated with MHS (King, Denborough and Zapf, 1972; Pollock and Britt, 1983). Only 5% of the MHS patients suffered from muscle cramps, pain or stiffness which have been stated to be common amongst MHS individuals (Pollock and Britt, 1983). One MHS patient had dystrophonia myotonica (DM). *In vitro* tests with normal results have been performed in patients with DM at other MH units (Moulds and Denborough, 1974; Brownell et al., 1983). Thus DM *per se* presumably does not affect the sensitivity to caffeine or halothane, and the diagnosis of MHS in this patient is considered appropriate. Three other neuromuscular diseases were encountered in our MHS patients: Duchenne muscular dystrophy, which has been increasingly reported in association with MHS (Brownell et al., 1983; Rosenberg and Heiman-Patterson, 1983), central core disease

which has a documented, high incidence of MHS (King, Denborough and Zapf, 1972; Frank et al., 1979) and an unspecified muscular dystrophy (Fletcher et al., 1982).

Creatine kinase (CK) was of little value in diagnosing MHS (fig. 7) even in families with fulminant MH. Sixty-two percent of MHS patients had increased CK concentration—a proportion similar to that reported by Ellis, Cain and Harriman (1978). However, in approximately 25% of the MH-negative patients without any muscular symptoms the concentration of CK was increased.

Our results of the *in vitro* contracture test for relatives are compatible with the theory that MHS is caused by a single autosomal dominant gene (King, Denborough and Zapf, 1972). The high proportion of MH negatives in the Danish families with possible MH is the result of reclassification of some families with possible MH after completion of the control biopsies.

ACKNOWLEDGEMENTS

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ERFORSCHUNG DER MALIGNE
HYPERTHERMIE IN DÄNEMARK UND SCHWEDEN

ZUSAMMENFASSUNG

In Dänemark wurden 1977 und in Schweden 1981 Zentren zur Erforschung der Empfänglichkeit für Maligne Hyperthermie eingerichtet. Zweihundert zehn Patienten aus 76 Familien wurden untersucht. Die Diagnose der Empfänglichkeit für Maligne Hyperthermie (MHS) wurde durch *in-vitro*-Exposition von Gewebe des M.vastus medialis auf Halothan oder Koffein gestellt. Die Kriterien für MHS in dieser Studie wurden nach Auswertung von 31 Kontrollbiopsien aufgestellt, die aus dem gleichen Muskel entnommen und denselben Anästhetika ausgesetzt waren wie die der MH-Patienten. Die Kriterien wurden also im Vergleich mit früher in dieser Zeitschrift vorgeschlagenen verändert. In unseren Laboratorien war der Halothan-Test (Exposition auf 0,5–2,0% Halothan) am empfindlichsten. Acht und achtzig Prozent der MHS-Patienten reagierten darauf. Der Koffein-Test war bei 68% der MHS-Patienten positiv, wobei Lösungen von 0,5–2,0 mmol Liter⁻¹ die beste Abgrenzung ermöglichten. Zwei und vierzig Prozent der MHS-Patienten reagierten nur auf einen der beiden Tests. Fulminante MH war der häufigste Grund für die Untersuchung. Alle Familien solcher Patienten wiesen MHS-Personen auf. Masseter-Spasmus als einziges Zeichen trat in 21 Familien auf, von denen 11 MHS waren. Nur 10% der MHS-Patienten hatten andere Zeichen oder Symptome neuromuskulärer Erkrankung wie z.B. Muskelkrämpfe oder Muskeldystrophie. In drei Familien war ein plötzlicher Kindstod (SIDS) aufgetreten, zwei Jugendliche einer MHS-Familie waren plötzlich gestorben, allerdings ohne Bezug zu einer Narkose.

INVESTIGATION OF MALIGNANT HYPERTHERMIA IN SWEDEN

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One hundred and thirty patients from 52 families were investigated for susceptibility to malignant hyperthermia (MH). The diagnosis of MH susceptibility (MHS) was made by in vitro exposure of muscle to halothane and to caffeine according to the protocol established by the European malignant hyperpyrexia group. Diagnostic criteria for the patients were established from examination of 13 normal control biopsies obtained from the same muscle and with the same anaesthesia as in the MH patients. These control results agree with and confirm the criteria formulated in the European MH group. The results are compared with our first 85 investigations performed prior to the establishment of the protocol. The main difference compared to the earlier material is the addition of an equivocal group (MHE), whose muscle reacted in vitro to either halothane or caffeine, but not both. All the families referred because of a fulminant MH reaction contained MHS or MHE members. There was a greater incidence of MH negative, families, suggesting an increased suspicion of MH amongst the clinicians.

KEY WORDS

Hyperthermia, malignant, in vitro testing.

Malignant hyperthermia (MH) is a rare, pharmacogenetic disorder which manifests itself during anaesthesia (1). Susceptibility to MH (MHS) can be determined by in vitro exposure of muscle to halothane (2) or caffeine (3). These in vitro muscle tests are used in many MH investigation units, but there was until recently a lack of agreement as to how the test should be performed or what diagnostic criteria to use. In 1983 the European malignant hyperpyrexia group was formed with the purpose of establishing a common in vitro test protocol and diagnostic criteria (4, 5). The group defined three categories of result for in vitro diagnosis: MH susceptible (MHS), normal (MHN) or equivocal (MHE). An in vitro test threshold was defined as an increase in muscle tension of 0.2 g or more. The diagnosis MHS implied a threshold to halothane at 2% or less plus to caffeine at 2 mmol l⁻¹ or less; MHE implies only one of the above thresholds; and MHN implies neither. This paper reviews 130 investigations performed according to this protocol and compares the result with 85 investigations performed prior to the protocol's establishment (6).

PATIENTS AND METHODS

Families were investigated if a member (the proband) exhibited signs associated with MH. The proband was investigated first; if he had not reached puberty, the parents were investigated. Relatives of positive families were then offered investigation. Clinical examination was performed with special regard to neuromuscular signs known to be associated with MH (7, 8). Creatine kinase (CK) concentration was measured before and after muscle biopsy in 62 patients.

Biopsy procedure

MH susceptibility was diagnosed by in vitro testing of muscle obtained from the vastus medialis muscle. Biopsies were taken under epidural anaesthesia (n=120) with 2% mepivacaine or under thiopentone, fentanyl and nitrous oxide in oxygen anaesthesia (n=10).

During anaesthesia, ECG and rectal temperature were monitored continuously and arterial pressure was measured intermittently. Patients found to be susceptible were observed for at least 24 hours.

The biopsy specimens for the in vitro test were transported and stored in Krebs solution at room temperature.

In vitro test procedure

For the in vitro test, pieces of muscle 15-25 mm long and 2-3 mm in diameter were dissected and suspended in a tissue bath with Krebs solution at 37°C and bubbled with preheated 5% carbon dioxide in oxygen (carbogen). Viability of the muscle specimen was demonstrated by supramaximal electrical stimulation at 0.2 Hz. The specimens were exposed to either halothane or caffeine in increasing concentrations, the muscle tension measured by a displacement transducer and a record obtained.

Two different tests were performed with halothane and caffeine respectively, one static and one dynamic. In the static test the muscle specimen was stretched initially to about 2 g and left for 10-20 minutes before adding halothane or caffeine. In the dynamic test the muscle was first allowed to rest at zero or low tension for three minutes and was then stretched at a rate of 4 mm min^{-1} for 1.5 minute to a tension of approximately 2 g, left for 1 minute and then shortened at the same rate

to the initial length. The whole cycle was thus 7 minutes (2, 6). After three such cycles halothane or caffeine was added. The concentrations of caffeine were 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 mmol l⁻¹. The nominal halothane concentrations were 0.5, 1.0, 2.0 and 3.0%, which corresponded to 0.11, 0.22, 0.44, 0.66 mmol l⁻¹ of dissolved halothane in the tissue bath. The dissolved halothane concentration was measured using a HPLC gas chromatography.

In the static test, the muscle was exposed to each concentration of caffeine or halothane until a plateau contracture was induced or 3 minutes had elapsed. In the dynamic test, exposure was for one cycle for each concentration.

Control biopsies were taken with informed consent from 13 healthy subjects during minor operation on the leg. The control patients were anaesthetized in the same way as the MH patients and the biopsies were all taken from the vastus medialis muscle. None of the control patients had experienced any anaesthetic complication nor was there any family history of neuromuscular disease.

For statistical comparison between groups a Mann-Whitney test was used. Values of $p < 0.05$ were considered significant.

Results

130 patients aged between 12 and 73 yr from 52 families were investigated. None developed signs of MH in the perioperative period. Figures 1 and 2 show the results from the control biopsies, which confirm the European criteria. It should be noted that in addition a dynamic caffeine test was performed (9). Whichever test, dynamic or static, gave the greatest increase in tension, was used to make the diagnosis.

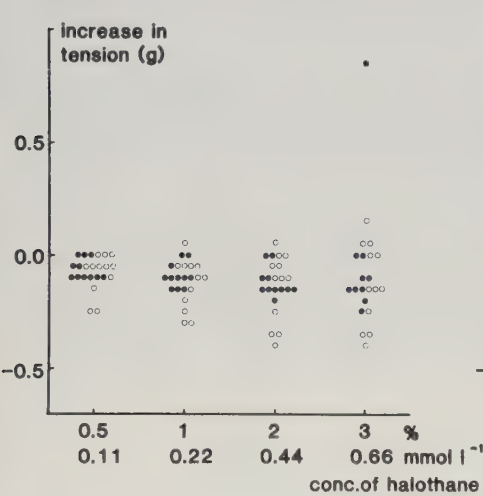


Fig. 1. Results from control biopsies in the static (●) and dynamic (○) halothane tests.

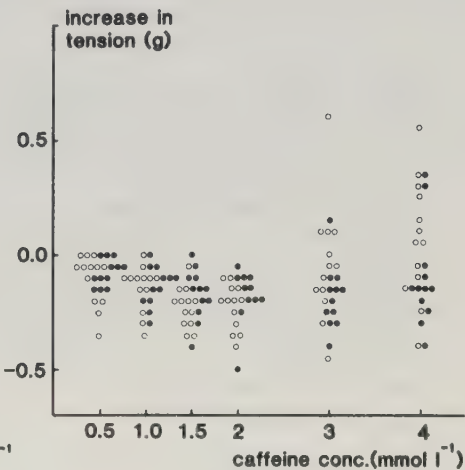


Fig. 2. Results from control biopsies in the static (●) and dynamic (○) caffeine tests.

The reasons for investigation and the results of the in vitro test of the patients are shown in table 1 and 2. The in vitro test from one patient was inconclusive and is not included in the figures. Twenty-seven patients reacted to both tests i.e. were MHS and 25 (MHE) patients reacted to one of the in vitro tests, in most cases to halothane. Spontaneous in vitro

contracture was noted in two patients; one was clearly MHS. The other patient had normal responses to both halothane and caffeine. She was the mother of a boy, not yet biopsied, who had experienced a possible MH reaction. The father had a caffeine threshold at 1.5 mmol l^{-1} i.e. he was diagnosed MHE. Previous experience causes us to believe that spontaneous contracture is typical of MH susceptibility, and therefore we have classed her MHE.

Table 1. Types of reaction leading to MH investigation. Number of probands biopsied in parenthesis. * Two or more of the following: tachycardia, arrhythmia, hypertension, rigidity, acidosis, increase in CK, myoglobinuria, fever.

Type of reaction	MHS	MHE	MHN
Fulminant MH reaction	5	2	0
Masseter spasm	2	4 (2)	9 (6)
Possible MH reaction*	5 (2)	7 (1)	15 (10)
Unexplained fever during or after anaesthesia	0	1 (1)	1 (1)
Central core disease	0	0	1 (1)
Total	12 (2)	14 (4)	26 (18)

Table 2. Results of in vitro tests of relatives of MHS and MHE probands. Relatives of MHE probands are indicated in parenthesis.

	MHS	MHE	MHN
Member of a family with fulminant MH	9	5 (2)	15 (3)
Member of a family with possible MH	15	4 (10)	14 (3)
Unexpected non-anaesthetic death (10)	1	0	0
Total	25	9 (12)	29 (6)

Table 3. Type of operation during which MH occurred or was suspected in the proband. Number of acute operations in parenthesis.

Type of operation	MHS	MHE	MHN
Abdominal surgery	3 (3)	0	5 (5)
Caesarian section	1 (1)	0	10 (5)
ENT surgery	6 (0)	7 (1)	6 (0)
Surgery for trauma	1 (0)	4 (3)	2 (1)
Eye surgery	0	0	0
Others	1 (0)	3 (0)	2 (0)
Total	12 (4)	14 (4)	25 (11)

The types of operation during which MH occurred or was suspected are shown in table 3. Age distribution and sex of the MHS and MHE probands are shown in figure 3. Five patients died from MH, four of these during acute surgery. Thus the overall mortality rate in this material was 19 %. The mortality rate in fulminant MH was 71 %. However, all the deaths in Sweden occurred before 1982 and one Swedish patient died in France 1983.

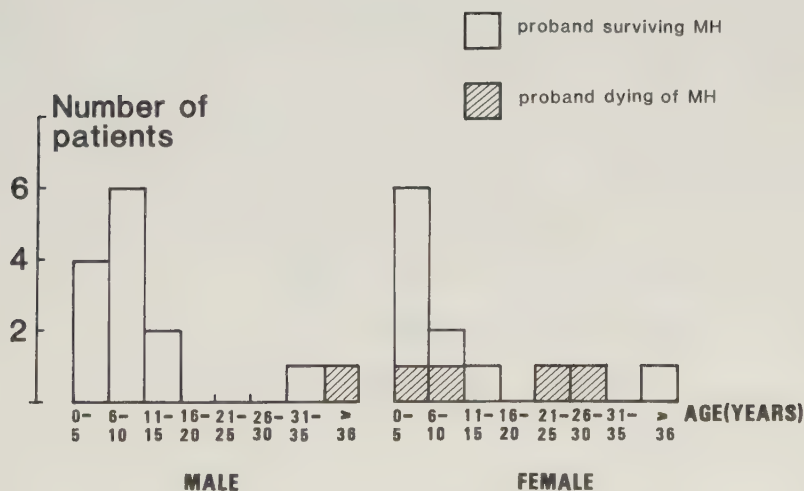


Fig. 3. Age and sex distribution of MHS and MHE probands.

History and clinical examination

No MHS patients had obvious neuromuscular disease but three had prominent lateral thigh muscles. One MHE patient had a very slight spastic tetraplegia and a Klippel-Feil deformity. Amongst the MHN patients one had central core disease (CCD), one sarcoidosis with involvement of peripheral nerves causing weakness of the legs, and one was born with a meningomyelocele causing paraplegia. The other patients were normal on clinical examination. 19 % of the MHS and MHE patients and 14 % of the MHN patients (n.s.) suffered from muscle cramps. 2 MHS patients (8.6 %) and 1 MHN patient (1.7 %) complained of muscular discomfort after drinking alcohol. 1 MHN patient (1.7%) complained of muscular discomfort after drinking coffee. There was considerable spread of values for CK, and the difference between MHS and MHN patients was significant ($p=0.001$) There was no significant difference between the preoperative and postoperative CK values in any group.

Discussion

The European MH group's diagnostic criteria are based on results of pooled control data from its members and on in vitro test results from survivors of a fulminant MH reaction. The main difference from our own earlier diagnostic criteria (6) is the addition of an equivocal group, which in the former material would have been regarded as MH susceptible. In the long term, diagnosis of MH susceptibility should become more certain as a result of this division. In practice patients proving to be MHE are told that they are susceptible. We have pooled the MHE and MHS results in order to compare the present results with others outside the European group.

The halothane test is still the most reliable in our hands since 89 % of the MHS and MHE patients had an abnormal response to halothane, but only 60 % to caffeine, which agrees with the group's experience (5). However, 9 % reacted only to the caffeine test which still therefore earns its place in the protocol. 40 % of patients investigated because of suxamethonium induced masseter spasm were found to be MHS or MHE. These figures are slightly less than in our earlier material (50%) and less than the 57 % reported by Rosenberg and Reed (11). We have also found a somewhat lower proportion of MHS and MHE patients in the group investigated because of a possible MH reaction; 44 % compared to 55 % in our earlier material. These figures suggest that in Sweden, as elsewhere, anaesthetists now are well aware of MH, leading to a greater frequency of referral, but also greater safety.

50 % of the MH reactions occurred during ENT surgery, a much higher figure than previously reported by us (6) or others (8). 19 % of the MH reactions were seen during surgery for trauma, which is in agreement with the

findings by Ellis and Halsall (12). Abdominal surgery including caesarian section was the third most frequent type during which MH occurred. A remarkably high proportion of negative results came from referrals because of a suspected MH reaction during caesarian section. However, many of these MHN patients were suffering from infection secondary to early rupture of the membranes and were thus reacting with symptoms resembling MH, such as a pyrexia, tachycardia and acidosis.

Except for ENT surgery which seems to carry a higher risk of MH, the likelihood of MH during other types of operations probably reflects the types of operations which are common amongst this age group. Mortality during acute surgery was 4 of 8 reactions, compared to one of 18 during elective surgery. Although the mortality rate in this material was 19%, no deaths due to MH have come to our knowledge since 1981. Remarkably few patients had any neuromuscular signs known to be associated with MHS (7, 8). The results of the in vitro contracture test from relatives of positive families confirm our earlier findings (6) and are compatible with the theory that MH susceptibility is caused by a single autosomal dominant gene (7). In contrast to others (8) we did not find a higher frequency of muscular cramps amongst MHS patients. As in our previous and in other's work (6, 13) CK proved to be an unreliable predictor of MH susceptibility. Alcohol is known to cause in vitro contracture of MHS muscle (2), but most MHS patients tolerated both alcohol and coffee well.

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STATIC VERSUS DYNAMIC IN VITRO TESTS IN THE DIAGNOSIS OF MALIGNANT
HYPERTHERMIA SUSCEPTIBILITY

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In vitro contracture tests, in which muscle specimens are exposed to halothane or caffeine, are at present the only generally accepted screening methods for the diagnosis of malignant hyperthermia susceptibility (MHS). Static tests, i.e. tests performed with the muscle held at constant length, are more common, but some MH investigation units also use dynamic tests, in which specimen length is varied. We have performed dynamic and static tests in parallel on muscle from one hundred and twelve patients. Our results demonstrate that the dynamic halothane test is more sensitive in discriminating between MHS and MH negative (MHN) individuals than the static halothane test. The dynamic caffeine test, however, is less sensitive in discriminating between MHS and MHN individuals, and nothing is to be gained by including it in the investigation protocol.

The most reliable method for establishing susceptibility to malignant hyperthermia (MH) is by in-vitro pharmacological testing of skeletal muscle (Ellis et al., 1972; Kalow, Britt and Richter, 1977; Moulds and Denborough, 1974). The diagnosis is made when muscle specimen tension increases by 0.2 g or more (a "threshold") on exposure to low concentrations of halothane and caffeine, in separate tests (European malignant hyperpyrexia group, 1984; Ørding, Ranklev and Fletcher, 1984). Although many different interpretations of the in-vitro tests are to be found, members of the European MH-group perform the in-vitro test according to a common protocol, and the diagnosis susceptible (MHS), negative (MHN) or equivocal (MHE) is made according to common criteria. The diagnosis MHS requires thresholds at less than 2% halothane and less than 2 mmol l⁻¹ caffeine; MHE implies positive response to only one drug.

Most MH-investigation units use static caffeine and halothane contracture tests. In these, the muscle specimen is maintained at a constant length whilst caffeine or halothane are added to the muscle bath. However in Great Britain, Denmark and Sweden a dynamic halothane contracture test (Ellis et al., 1978) is used as well. Here the muscle specimen is subjected to a regular cycle of stretching and relaxation during pharmacological exposure (Ellis et al, 1978; Ørding, Ranklev, and Fletcher, 1984). No description of a dynamic caffeine contracture test has been published, although it has been performed in Great Britain, Denmark and France (Ellis, Ørding, Reiss- Kozack, and Moeller, personal communications) as well as in our own unit. This paper describes our findings when static and dynamic tests were performed in parallel.

PATIENTS AND METHODS

One hundred and twelve patients were investigated for susceptibility to MH. Eighteen patients were survivors of a suspected MH-reaction; ninety-two were relatives of known or suspected MH-probands. One patient had known central core disease, and one was a father of two teenage boys who died suddenly without any explanation (Ranklev, Fletcher and Krantz, 1985).

In addition, normal control biopsies were taken from twelve healthy subjects, during minor surgery on the leg. All muscle biopsies, including the controls, were taken from vastus medialis during epidural analgesia with mepivacaine or during thiopentone, fentanyl, N₂O/O₂-anaesthesia.

For the in vitro test, pieces of muscle 15 - 25 mm long and 2 - 3 mm in diameter were dissected and suspended in a tissue-bath which was perfused with Krebs solution at 37°C and bubbled with preheated 5 % carbon dioxide in oxygen. Specimen viability was demonstrated by supramaximal stimulation at 0.2 Hz. The specimens were exposed to either halothane or caffeine in increasing concentrations, and the muscle tension was measured by a displacement transducer and recorded on paper (European malignant hyperpyrexia group, 1984; Ørding, Ranklev and Fletcher, 1984).

In the dynamic test the muscle was first allowed to rest at zero or low tension for three minutes, and was then stretched at rate of 4 mm min⁻¹ for 1.5 min to a tension of approximately 2 g, left for 1 min and then shortened at the same rate to the initial length. The whole cycle was thus 7 min. (Ellis et al., 1978; Ørding, Ranklev and Fletcher, 1984). After three such cycles halothane (0.5, 1, 2 and 3 %) or caffeine (0.5, 1, 1.5, 2, 3 and 4 mmol l⁻¹) were added for one cycle each. In the static test the muscle was stretched to approximately 2 g and left for

20-30 min before adding halothane or caffeine in the same concentrations as in the dynamic test. Each concentration was continued until a new plateau contracture was induced or 3 min had elapsed.

The criteria for susceptible, equivocal or negative results are as agreed by the European malignant hyperpyrexia group (1984), except that the dynamic caffeine test also was taken into account. The test giving the greatest increase in tension was used to make the diagnosis. The tests were performed in randomized order. For statistical comparison in each group a Student's t-test was used.

RESULTS

In the controls (12 in a former material (Ørding, Ranklev and Fletcher, 1984) and 12 in the present), no specimen demonstrated a contracture when exposed to 2 % or less halothane or 2 mmol or less caffeine.

25 patients were diagnosed as MHS, 20 MHE and 67 MHN. In both the MHS-group ($p = 0.008$) and the MHE-group ($p = 0.002$), thresholds with halothane were obtained at lower concentrations in the dynamic test than in the static. Five MHS-patients and all but two MHE had normal static halothane tests but abnormal dynamic tests. Five patients in the MHE-group but none in the MHS-group had normal dynamic halothane tests (fig 1).

In the caffeine test, thresholds were obtained at significantly lower concentrations in the dynamic than in the static test in both the MHE ($p = 0.004$) and MHN-groups ($p = 0.006$). However no significant difference could be seen between the static and dynamic caffeine tests in the MHS group ($p = 0.5$)(fig 2).

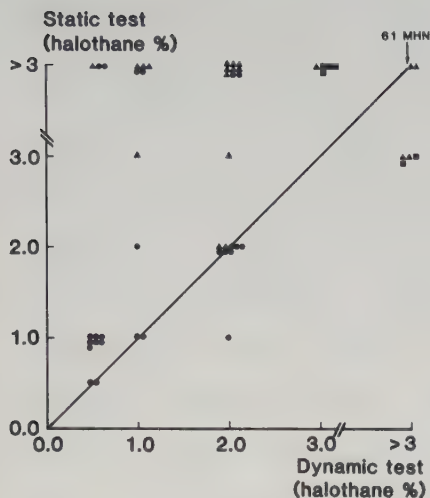


Fig. 1. Thresholds in dynamic v. static halothane tests in 25 MHS (), 20 MHE () and 67 MHN () patients.

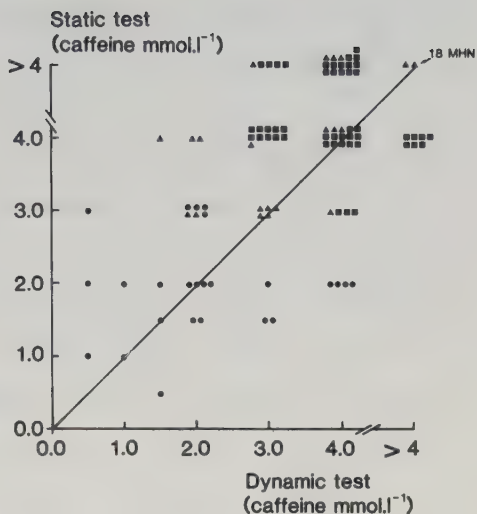


Fig. 2. Thresholds in dynamic v. static caffeine tests in 25 MHS (), 20 MHE () and 67 MHN () patients.

DISCUSSION

In the establishment of MH susceptibility, the only procedure which has stood the test of time is in-vitro pharmacological muscle testing. Nevertheless the method has not been fully satisfactory because of the dearth of acceptable control data: i.e. results from vastus muscle obtained in good condition from healthy individuals anaesthetised in the same way as patients undergoing MH investigation. Thus criteria have to some extent been defined arbitrarily.

It is gratifying that results from normal control muscle have so far upheld the criteria established by the European MH-group (1984). However, more controls are necessary for greater diagnostic safety and for the further clarification of the status of the MHE group, most of whom would

have been classified as MH susceptible prior to the formation of the European MH group.

The present results demonstrate that the dynamic halothane test is more sensitive in discriminating between MHS and MHN patients than the static halothane test; if the static halothane test alone were used many MH susceptible patients could be misdiagnosed, apparently having only a pathological caffeine test. Regardless of which criteria for MHS are chosen within reason, it is still true to say that the dynamic halothane test is the more sensitive, since the overwhelming majority of points in fig 1 are on or above the line of identity. However, with caffeine, thresholds were obtained at significantly lower concentrations in the dynamic than in the static test in the MHN-group, whilst there was no difference in the MHS-group. The dynamic caffeine test is thus of less value in discriminating between MHS and MHN patients than the static caffeine test.

The results from the MHE-group are more difficult to interpret. Only four MHE-patients had a pathological contracture with caffeine (and only in the dynamic test) but not with halothane. It is impossible to ascertain their true status from the history and pedigree. The other 14 MHE-patients had normal response to caffeine in both tests. Because of their pedigree at least two of these must be susceptible. Both have a pathological contracture to dynamic but not static halothane tests and would have thus been diagnosed as MHN without the use of the dynamic test.

At present the European consensus of opinion is that both the halothane and the caffeine test are necessary to diagnose MH susceptibility, although this opinion may be revised when more control data becomes

available. Both static and dynamic halothane tests are considered necessary, as our results confirm. However, nothing would appear to be gained by including a dynamic caffeine test in the protocol.

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MICROCALORIMETRIC STUDIES IN MALIGNANT HYPERPYREXIA SUSCEPTIBLE INDIVIDUALS

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The only generally accepted method of diagnosing susceptibility to malignant hyperpyrexia (MHS) is *in vitro* testing of muscle (Kalow, Britt and Richter, 1977; Ellis et al., 1978). However, since this is a time-consuming technique which requires an open muscle biopsy, a simpler method of diagnosis would be of value.

Although the primary manifestation of malignant hyperthermia (MH) is an increase in heat production in muscle, platelets also contain contractile elements and have been the focus of attention. Indeed, abnormalities of platelet metabolism and aggregation have been reported (Solomons, Tan and Aldrete, 1978; Zsigmond, Penner and Kothary, 1978) in association with MH.

We have measured *in vitro* the heat produced by exposing small specimens of muscle to halothane and by exposing platelets to halothane and caffeine.

PATIENTS AND METHODS

Muscle preparation

A muscle biopsy was taken from 26 individuals being investigated for susceptibility to MH, and from seven normal controls during other operations. MH-suspected individuals were identified as MH susceptible (MHS), or MH negative (MHN) after the *in vitro* exposure of the muscle to halothane and to caffeine (Ørding, Ranklev and Fletcher, 1984). All muscle biopsies from MH-suspected individuals were from the vastus medialis and included the motor point. The biopsies from the control patients were taken from the rectus abdominis, gluteus maximus or gastrocnemius. Anaesthesia for the biopsies was either femoral nerve blockade or thiopentone-fentanyl-nitrous oxide in oxygen.

After excision the muscle was stored and

SUMMARY

In an attempt to develop a diagnostic test for malignant hyperpyrexia (MH) without the need for open muscle biopsy, we have investigated the change in heat production brought about when platelets and muscle are exposed to halothane and caffeine. Halothane 3% caused platelet aggregation, which was itself associated with heat production. Caffeine 4 mmol litre⁻¹ decreased heat production in platelets from both MH susceptible and non-susceptible individuals. Muscle exposed to 4% halothane showed an increase in heat production in both types of tissue. There were no significant differences in heat production between MH-susceptible and non-susceptible tissue in any of the tests.

transported at 4 °C in Krebs-Ringer phosphate (KRP) buffer solution containing glucose 8.25 mmol litre⁻¹ and insulin 0.1 iu ml⁻¹. The fibres were dissected to about 1 mm diameter (about 50 mg weight) and placed in the calorimeter within 30 min of excision.

Platelet suspensions

Venous blood samples were collected, from 14 individuals being investigated for MH sensitivity, in 10-ml Vacutainer tubes containing 25 U.S.P. units of sodium heparin (Kabi, Sweden) and 5% Dextran 80 solution 2 ml (Pharmacia, Sweden). Platelet rich plasma (PRP) suspensions were prepared as described by Levin (1971). The concentration of platelets in the cell suspensions was about 150×10^9 litre⁻¹. Duplicate platelet and leucocyte counts were made by phase contrast microscopy. No erythrocytes were observed in the cell suspensions used.

Calorimetric measurements

The studies on muscle were performed in a perfusion calorimeter (Wadsö, 1974) in which the

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fluid was maintained at 37 °C by a heat exchanger to ensure minimal heat loss from the system. Heat production was measured as the change in voltage produced in a thermopile placed round the calorimeter vessel. After 30 min the perfusion fluid was changed to one equilibrated with 4% halothane.

The studies on platelets were performed in microcalorimeters of the thermopile heat conduction type (Spink and Wadsö, 1976). The samples were enclosed in 1.0-ml stainless steel ampoules. Measurements were made under static conditions at 37 °C as described by Monti and Wadsö (1977). Two samples were measured simultaneously. Corrections were made for the heat production of leucocytes in the PRP suspensions (2% of the total heat production (Bandmann, Monti and Wadsö, 1975)) and for the final pH to pH 7.4 (Monti and Wadsö, 1977). The coefficient of variation for the method has previously been determined to be 4% (Monti and Wadsö, 1977).

In the caffeine-platelet study, caffeine diluted in KRP-buffer was added to PRP to a final concentration of 4 mmol litre⁻¹. The control ampoule contained PRP and KRP buffer solution. Samples from nine patients (five MHS and four MHN) were tested. In the halothane-platelet study, platelets were suspended in autologous plasma and the reactant ampoule was equilibrated with 3% halothane for 10 min before measurements were begun. Samples were obtained from one MHS and two MHN patients. In addition, two equivocal patients (MHE (European MH group, 1984)) were tested.

Statistics

Differences were tested by the Mann-Whitney test for unpaired data.

RESULTS

Effect of halothane on heat production in muscle

Figure 1 shows a typical muscle calorimetry tracing. Before the addition of halothane there was a continuous decrease in heat production, possibly reflecting a decrease in the availability of substrate in the cells. The addition of halothane was, in all instances, followed by an increase in the rate of heat production; on many occasions the increase was enough to reverse the trend.

Heat production was measured as the area under the "hump" in the tracing (the shaded area in

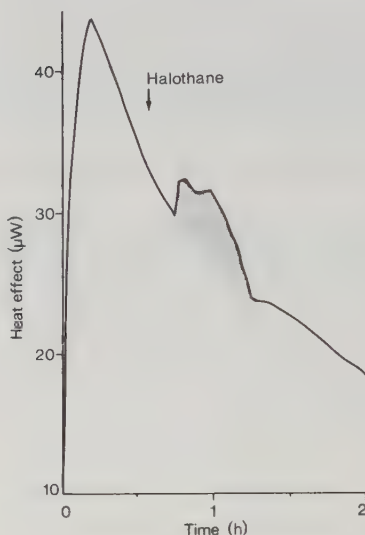


FIG. 1. Microcalorimetry tracing showing effect of halothane on muscle metabolism.

figure 1). There were no significant differences between MHS ($n = 12$), MHN ($n = 14$) and normal controls ($n = 7$).

Effect of caffeine on heat production by platelets

Heat production was determined with and without the addition of caffeine to a final concentration of 4 mmol litre⁻¹. In all instances heat production decreased in the suspensions containing caffeine. There were no significant differences between MH-positive and MH-negative patients.

Effect of halothane on heat production by platelets

In four patients (one MHS, two MHN and one MHE) halothane caused an increase in heat production; in one equivocal patient heat production decreased.

DISCUSSION

Measurement of heat production in cells by calorimetry gives information about total cellular metabolic activity. The present method of muscle fibre preparation has been used extensively by other investigators (Lundholm et al., 1975) who

were unable to demonstrate morphological or functional alterations in the specimens (Bylund et al., 1976). Muscle for this test was excised and tested at the same time as the muscle used for the *in vitro* halothane and caffeine tests, during which satisfactory viability was demonstrated by repeated electrical stimulation. In the model described above, halothane increased the metabolism of muscle tissue, but there was no significant difference between MHS and MHN individuals. The muscle specimen was not stretched during the test. In the *in vitro* test for MH sensitivity, a halothane contracture cannot usually be demonstrated at zero tension. It may, therefore, be necessary to stretch the muscle to 1–2 g tension in order to observe a difference between MHS and MHN individuals.

Halothane had a variable effect on heat production by platelets, increasing it in four patients and decreasing it in one. Although the MHS patient showed the greatest increase in heat production, the differences were too small to be of any diagnostic value. More importantly, halothane has a tendency to make platelets aggregate. Because aggregation *per se* causes an increase in heat production by platelets (Ross, Fletcher and Jamieson, 1973) we do not believe that this method can have any diagnostic value.

Caffeine, on the other hand, decreased heat production in both MHS and MHN platelets, although the effect of caffeine on the intact organism is to increase heat production (Acheson et al., 1980). Other xanthine derivatives have been shown to have the same effect on platelets (Monti and colleagues, in preparation).

We conclude that the microcalorimetric methods described above cannot be used to distinguish between tissues from MHS and MHN individuals.

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CLINICAL AND MUSCLE BIOPSY FINDINGS IN RELATION TO MALIGNANT HYPERTHERMIA SUSCEPTIBILITY

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155 patients were investigated for malignant hyperthermia susceptibility (MHS), by in vitro testing of muscle taken from the vastus medialis muscle. Histopathological and histochemical investigation of muscle was also performed. 90% of the patients replied to a questionnaire concerning present or previous neuromuscular symptoms. The majority of MHS and MH negative (MHN) patients had no or only minor histopathological and histochemical abnormalities. Core-targetoid fibres were the only potentially important abnormalities found exclusively in MHS patients. There were no differences in neuromuscular symptoms between MHS, MHN and control patients, and most patients in both the MHS and MHN group were normal on clinical examination.

Key words: Hyperthermia, malignant, muscle biopsy, muscle histochemistry,
muscle histopathology, myopathy.

Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle characterized by a fulminant hypermetabolic response to certain agents. It usually presents during anaesthesia after triggering by inhalational anaesthetics or depolarizing muscle relaxants. Some MH affected individuals have obvious musculoskeletal abnormalities (1) or myopathies (2, 3, 4). Previous reports have described MH susceptible (MHS) individuals as sometimes exhibiting musculoskeletal disorders. Scoliosis, patellar luxation, muscle cramps and early fatigue with muscular effort have been reported in association with MHS (5, 6, 7). Increased frequencies of inguinal hernia, strabismus or ptosis have also been described. The occurrence of a special MH-myopathy is, however, controversial. This paper describes the clinical and histopathological findings in 155 patients referred for screening of MHS.

MATERIAL AND METHODS

84 patients suspected of being susceptible to MH were investigated by in vitro muscle testing as described by Ørding et al. (8). A further 71 patients were investigated according to the The European MH-group protocol (9). The patients' ages ranged from 13 to 66 years. They were diagnosed as positive (MHS), negative (MHN) or equivocal (MHE). All patients were screened with respect to neuromuscular signs and symptoms. A more detailed neurological examination was performed in 36 patients, which included manual testing of muscle strength. The result of this examination was expressed as normal or abnormal. All patients received a questionnaire, concerning present or previous neuromuscular symptoms. The patients were asked to specify positive symptoms.

Control biopsies were taken from the vastus medialis muscle of healthy subjects during minor surgery of the leg. They received the same anaesthesia as the patients being screened. 12 control biopsies were performed according to Ørding et al. (8) and 13 according to the European MH-group (9).

Muscle biopsies were taken from the vastus medialis muscle. Muscle pieces about 10 x 3 x 3 mm were placed immediately after excision in 4% neutral buffered formalin for histopathological investigation. For histochemical investigation muscle pieces about 10 x 3 x 3 mm were immediately after the excision frozen in a mixture of iso-pentane (BDH Chemicals Ltd, Poole, G.B.) and dry ice. The frozen pieces were then stored in a refrigerator at -70°C until analysed. Histopathological examination of 155 muscle biopsies was performed and 150 biopsies were examined with the histochemical method.

Paraffin-embedded material was stained with hematoxylin and eosin, van Gieson, and Ladewig staining. Frozen material was stained for the following enzymes: myofibrillar adenosine triphosphatase (ATP-ase) with preincubation at pH 9.4 and pH 4.6. NADH-diaphorase (NAD: nicotinamideadenine dinucleotide) (10), phosphorylase (11) and acid phosphatase. Periodic acid Schiff (PAS) reaction was done for glycogen. Oil-red-O staining was used for lipids. Hematoxylin-eosin, Weigert HTX-van Gieson, and modified Gomori trichrome staining (12) were also done on the frozen material.

The areas of type 1 and type 2 fibres were calculated using a digitizer (Bausch and Lomb, Houston Instrument) connected to a computer. (Type 1 fibres were defined as those fibres staining lightly for ATPase at pH 9.4 and type 2 fibres as those staining darkly). Determination of the frequency of the different fibre types and measurements of the fibre area was performed in 9 MHS patients and in 10 MHN patients. These biopsies were randomly selected from the whole material. The measurements were made in the area of the biopsy where the best cross-sections of fibres were found. All fibres were measured in the selected area.

For the quantitative analysis of muscle biopsies a Student's unpaired t-test was used. For statistical comparison between groups a Fisher's exact test was employed. The MHS and MHE group was pooled, when the statistical evaluation was employed, in order to compare our results with others not applying the criteria of the European MH group.

RESULTS

53 patients were diagnosed MHS, 10 MHE and 92 MHN. Only a minority of patients complained of any symptoms which might have been of neuromuscular origin (table 1). Amongst the MHS-patients only one with central core disease (CCD) and one with myotonic dystrophy had obvious neuromuscular disease. One patient had a suprapatellar muscular atrophy and three patients had prominent lateral thigh muscles. The rest of the MHS patients were normal on clinical examination.

Table 1 Neuromuscular symptoms reported to be associated with MH susceptibility in MHS, MHE, MHN and control patients. Evaluation of the replies to the questionnaire. * = Frequently occurring spontaneous painful muscular cramps. ⁰ = Subjective feeling of muscle weakness when climbing stairs, carrying shopping bags etc.

History or signs of:	MHS (n=50)	MHE (n=7)	MHN (n=83)	Controls (n=19)
Strabismus	2 (4%)	0	6 (7%)	1 (5%)
Inguinal hernia	4 (8%)	2	4 (5%)	3 (16%)
Kypho-scoliosis	2 (4%)	2	6 (7%)	0
Patellar luxation	5 (10%)	1	3 (4%)	1 (5%)
Muscle cramps*	11 (22%)	0	13 (16%)	3 (16%)
Muscle weakness ⁰	6 (12%)	1	4 (4%)	0

Amongst the MHN patients was one with muscular weakness of late onset in whom core-targetoid fibres had been found in a previous muscle biopsy, another had sarcoidosis with peripheral neuropathy causing weakness of the legs, and one a meningocele causing weakness of the legs. One MHE patient had a slight spastic tetraplegia and a Klippel-Feil deformity. 140 patients (90%) replied to the questionnaire. Amongst these patients 50 were diagnosed MHS, 7 MHE and 83 MHN (table 1). 19 control patients (76%) answered the questionnaire. There were no differences in incidence of any symptoms between the groups.

The findings in the muscle biopsies are presented in table 2. Stains for lipids, phosphorylase and glycogen were normal in all biopsies. As seen in the table there are no changes in the MHS group of diagnostic significance. It is, however, of interest that core-targetoid changes were found only in the MHS group, one of whom had the clinical diagnosis of CCD.

The results of measurements of frequency of type 1 and type 2 fibres and of muscle fibre area are presented in table 3 and 4. There were no differences between biopsies from MHS and MHN patients in frequency of type 1 and type 2 fibres or in fibre area. The only significant difference was that the coefficient of variation of type 1 area was higher in the negative group ($p < 0.01$).

Table 2 Muscle biopsy findings in MHS, MHN and MHE patients.
Some patients had more than one type of change.

Findings	MHS (n = 53)	MHN (n = 92)	MHE (n = 10)
Muscle fibre degeneration	3 (1 pat with myotonic dystrophy, 1 pat with CCD)	4 (single fibres)	-
Muscle fibre regeneration	3 (in 2 only a few fibres)	1 (in only a few fibres)	-
Inflammatory infiltrates	1	11 (in 10 the infiltrates were discrete)	-
Internally placed nuclei in a few fibres	27 (51%)	43 (47%)	7
Internally placed nuclei in several fibres	3 (1 pat with myotonic dystrophy, 1 pat with CCD)	-	-
Increase of connective tissue	2 (slight in one)	-	-
Atrophic fibres	36 (in 30 only a few fibres) (68%)	59 (in 52 only a few fibres) (64%)	7 (in 6 only a few fibres)
Uneven distribution and/or grouping of type 1 and/or type 2 fibres	1	8	-
Variation of fibre size	40 (22 more pronounced of type 1 fibres 11 more pronounced of type 2 fibres) (75%)	84 (52 more pronounced of type 1 fibres 21 more pronounced of type 2 fibres) (91%)	10 (3 more pronounced of type 1 fibres, 2 more pronounced of type 2 fibres)
Fibre type predominance	4 (predominantly type 1: one patient with CCD)	-	-
Changes in the intermyofibrillary network (e.g. moth-eaten fibres)	5 (9%)	3 (single fibres with ragged red appearance) (3%)	-
Cores	3 (one patient with CCD)	-	-
Splitting	6 (11%)	10 (11%)	1

Table 3 Frequency of type 1 and type 2 fibres in MHN patients.

M: Male
F: Female

Age	Sex	Numbers of fibres meas.	Frequency of type 1 fibres	Frequency of type 2 fibres	Area μm^2 Type 1	Area μm^2 Type 2
48	F	110	62%	38%	4009	2490
					SD 1277	1101
35	M	65	48%	52%	7030	7211
					SD 1892	1254
35	M	48	54%	46%	6868	10089
					SD 2620	2392
35	M	164	37%	63%	2811	2751
					SD 846	814
29	F	96	43%	57%	5125	3802
					SD 1366	742
25	F	98	51%	49%	3770	4490
					SD 1087	878
22	M	73	59%	41%	4754	7593
					SD 1602	882
21	F	92	51%	49%	4219	5023
					SD 1428	867
23	F	66	42%	58%	5988	6460
					SD 1882	1026
51	M	72	50%	50%	4936	7214
					SD 1164	1051
Mean 32,4 SD 10,6		Mean 88 SD 33	Mean 49,7 SD 7,7	Mean 50,3 SD 7,7	Mean 4951 SD 1356	Mean 5712 SD 2398

Table 4 Frequency of type 1 and type 2 fibres in MHS patients. M: Male
F: Female

Age	Sex	Numbers of fibres meas.	Frequency of type 1 fibres	Frequency of type 2 fibres	Area μm^2	
					Type 1	Type 2
53	F	65	51%	49%	6704	5456
					SD 1177	1276
34	M	76	41%	59%	4815	6716
					SD 750	1399
36	M	91	57%	43%	5431	4447
					SD 1167	811
32	M	98	46%	54%	4780	4228
					SD 804	1032
32	M	78	32%	68%	5376	5788
					SD 1155	1510
29	F	137	42%	58%	3576	3270
					SD 752	883
19	M	119	59%	41%	3513	4399
					SD 900	861
23	F	59	73%	27%	6606	6715
					SD 2297	1077
18	F	90	40%	60%	4428	5761
					SD 1237	852
Mean 30,7 SD 10,6		Mean 90 SD 25	Mean 49% SD 12,4	Mean 51 SD 12,4	Mean 5025 SD 1143	Mean 5198 SD 1183

DISCUSSION

Harriman (13) presented a series of 200 biopsies from persons who had been subjected to the *in vitro* test. All biopsies were, as in our study, taken from the vastus medialis muscle. 120 biopsies from MHN persons were examined, of which half showed some abnormalities. In 35 biopsies of 80 from MHS patients pathological changes were found. Harriman concluded that there are changes that are characteristic of MH-myopathy: increased internal nuclei, moth-eaten fibres and core-targetoid fibres, changes which are not, however, often observed in asymptomatic MHS-patients. Our own study suggests, however, that with the possible exception of fibres with core-targetoid changes there are no findings that are characteristic or diagnostic of MH-myopathy. In our study there were three MHS patients with core-targetoid fibres in the muscle biopsy and they are described below.

Target fibres are defined as muscle fibres with rounded areas devoid of staining for oxidative enzymes, and with three concentric zones - one with no staining, one with increased staining and one with normal staining. The fibre is targetoid when the zone with increased staining is absent or indistinct. Cores are defined as almost circular areas with no staining for oxidative enzymes (the core is sometimes surrounded by a dense rim). It is sometimes difficult to differentiate between target, targetoid and core changes. The term core-targetoid fibres was used by Harriman (13), in the above mentioned study, and is also used in this study. Target fibres and targetoid fibres are also found in muscle biopsies from patients with neuropathy. Patients with neuropathy having target or targetoid fibres

have not to our knowledge been shown to be MHS. Core-targetoid fibres may indicate MHS only when they are found in biopsies without signs of neuropathy.

The first patient with core-targetoid fibres had also the clinical diagnosis of central core disease. The patient was a 47 year old woman with slowly progressive muscular weakness since early childhood. She had been anaesthetized twice without developing MH symptoms, but had a positive in vitro test (fig. 1). The second MHS patient found to have core-targetoid fibres had no clinical symptoms of neuromuscular disease (fig. 2). He was the 16 year old brother of a girl who died of acute MH. The biopsy from the third patient, a 32 year old man, is of special interest. The biopsy was taken 3 months after acute MH. Core-targetoid and moth-eaten fibres were found only in one part of the biopsy next to an area of severe muscle fibre destruction (fig. 3). In other parts of the biopsy only minimal changes were found, and in these areas there were no core-targetoid changes. These examples indicate that the changes in the muscle that can lead to MH also can lead to the development of core-targetoid changes and that these changes can be associated with MH regardless whether they occur in muscle from patients with central core disease or those with no overt neuromuscular disease. It should be observed, however, that one patient in the MHN group had muscular symptoms and core-targetoid changes in a previous muscle biopsy.

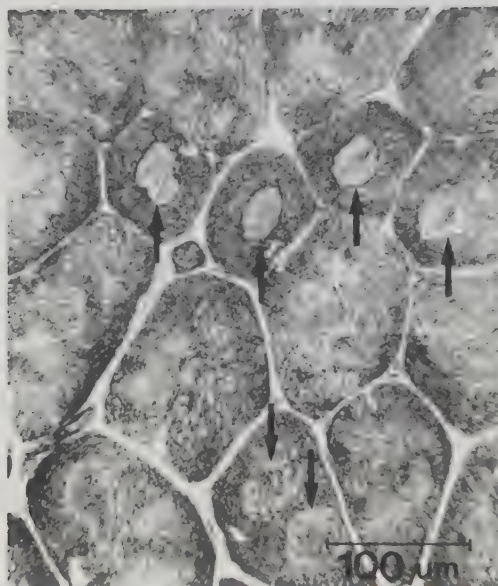


Fig. 1 Biopsy from a 47 year old woman with central core disease. Medial vastus muscle. Staining for NADH-diaphorase. Arrows show core-targetoid changes.

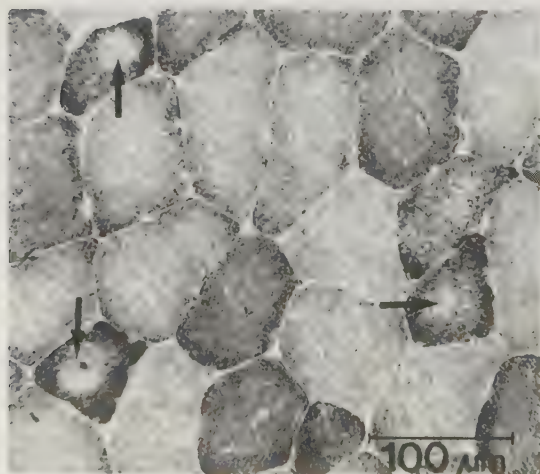


Fig. 2. Biopsy from a 16 year old boy. No neuromuscular symptoms. Medial vastus muscle. Staining for NADH-diaphorase. Arrows show core targetoid changes.

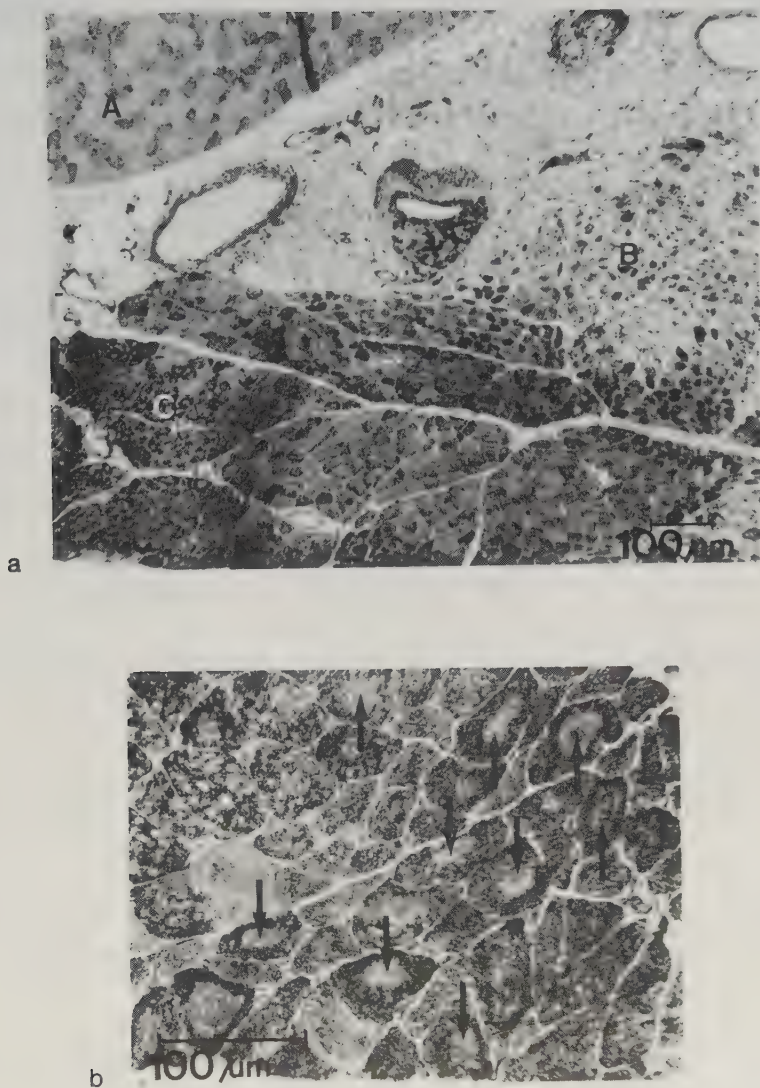


Fig. 3. Biopsy from a 32 year old man taken 3 months after an attack of MH. Staining for NADH-diaphorase. 3 a) At A normal muscle. At B fibrosis and severe destruction of muscle tissue. At C fibres with core-targetoid changes. These changes are shown at higher magnification in fig. 3 b.

The clinical investigation and the questionnaire did not confirm that there were any differences between the groups in the frequency of squint, hernia, kyphoscoliosis, patellar luxation, muscular weakness or muscle cramps.

We conclude that most MHS subjects do not have a specific myopathy. They are healthy individuals with a similar incidence of minor muscular symptoms and microscopic changes to normals. Our results indicate that in vitro testing for MHS should be considered if there are core-targetoid fibres in muscle biopsies, without clinical or microscopic signs of neuropathy. A histochemical and histopathological investigation could, however, not be used as a screening for MH susceptibility.

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